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CONTENTS

Determination of Nitrogen in Organic Compounds without Distillation. Part II.—Determination of Nitrogen in Nitro, Nitroso and Azo Compounds.....Muhammad Ashraf, M.K. Bhatty and R.A. Shah 135

Studies on Lavandula Stoechas Linn. (Labiatae). Part I.....S. Fazal Hussain and Georg Hahn 137

Development of Rapid Methods for the Estimation of the Oil Content of Single Cottonseeds. Part IV.—Investigation of an Electrical Circuit for Measuring the Electrical Response of One Cottonseed at 1,000 Cycles/Second.....A.K. Bhuiya, S. Hilal Ahmad and Mazhar M. Qurashi 143

On the Radio-Frequency System of a 30 Mev Microtron. Part II.....M. Innas Ali 149

Unsaponifiables in Fats: Quantitative Determination.....M. Qudrat-i- Khuda, H. C. Das and N.A. Khan 156

Cereals and Cereal Products: Properties of Certain Starch Varieties and their Sources in East Pakistan.....M. Qudrat-i-Khuda, B.D. Mukherjee, M.A. Hossain and N.A. Khan 159

A Study of Watermelon Rust.....S.R.H. Rizvi and S.Z. Hasanain 163

A Study of the Atmospheric Fungal Flora of Karachi Cantt.....S. Iftikhar Ahmad, M. Sayeed Quraishi and S. M. Murtuza 169

Some Interesting Strains of Aspergillus Ochraceous Group.....S. Iftikhar Ahmad and M. Sayeed Quraishi 172

Short Communications :

STUDIES IN THE RELATIONSHIP BETWEEN VISCOSITY AND MOLECULAR STRUCTURE. PART VI.—EVIDENCE FOR FINE STRUCTURE IN THE STEPS OBSERVED IN THE ENERGY OF ACTIVATION OF VISCOUS FLOW IN ETHYLENE GLYCOL.....A.K.M. Ahsanullah, S. Rahmat Ali and Mazhar M. Qurashi 175

TWO NEW SPECIES OF GENUS CURVULARIA. CURVULARIA SIDDIQUI SP. NOVO AND CURVULARIA ELLISI SP. NOVO.....S. Iftikhar Ahmad and M. Sayeed Quraishi 177

RESTING HABITS OF ANOPHELES SPECIES IN MYMENSINGH DISTRICT, EAST PAKISTAN..M. Sayeed Quraishi 178

Book Notices 181

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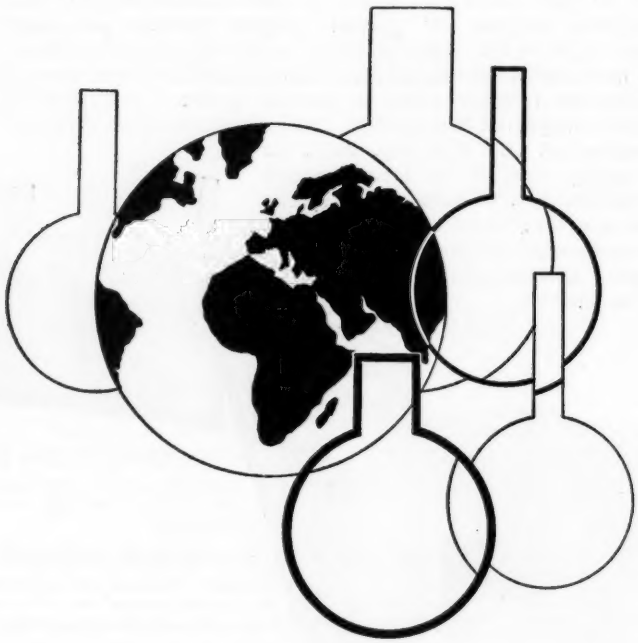

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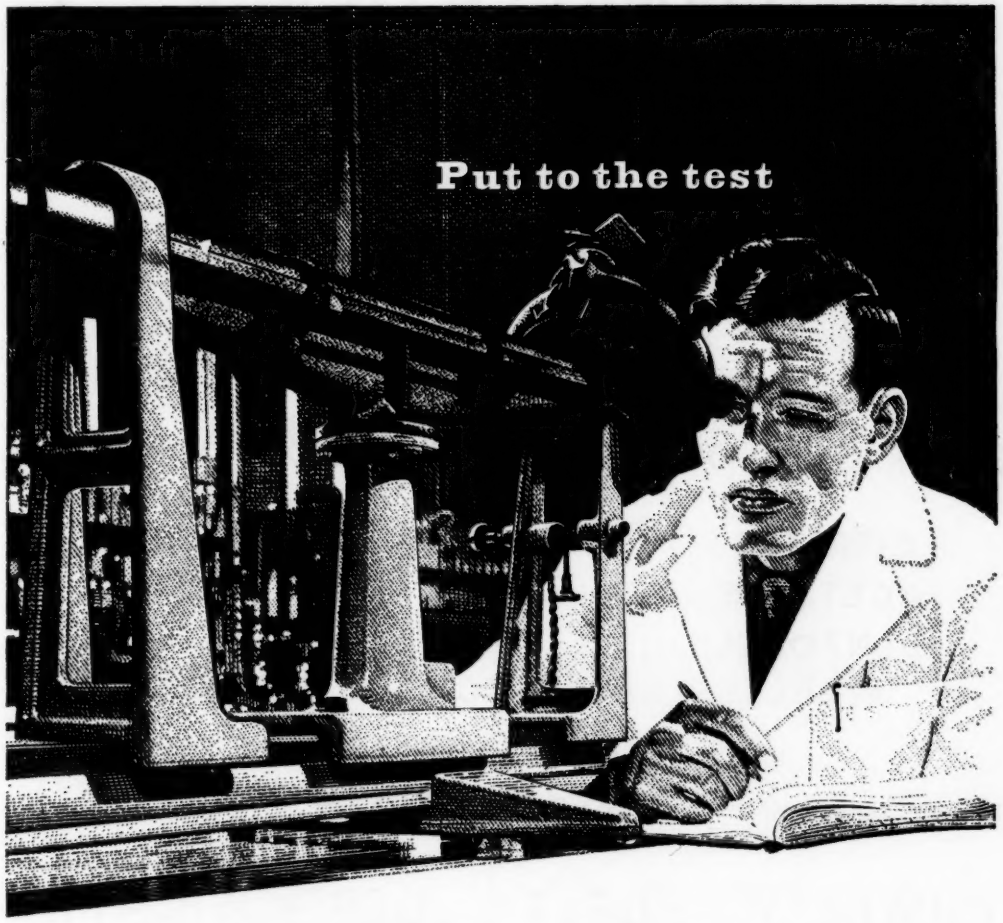

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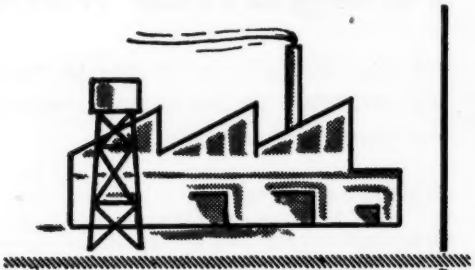

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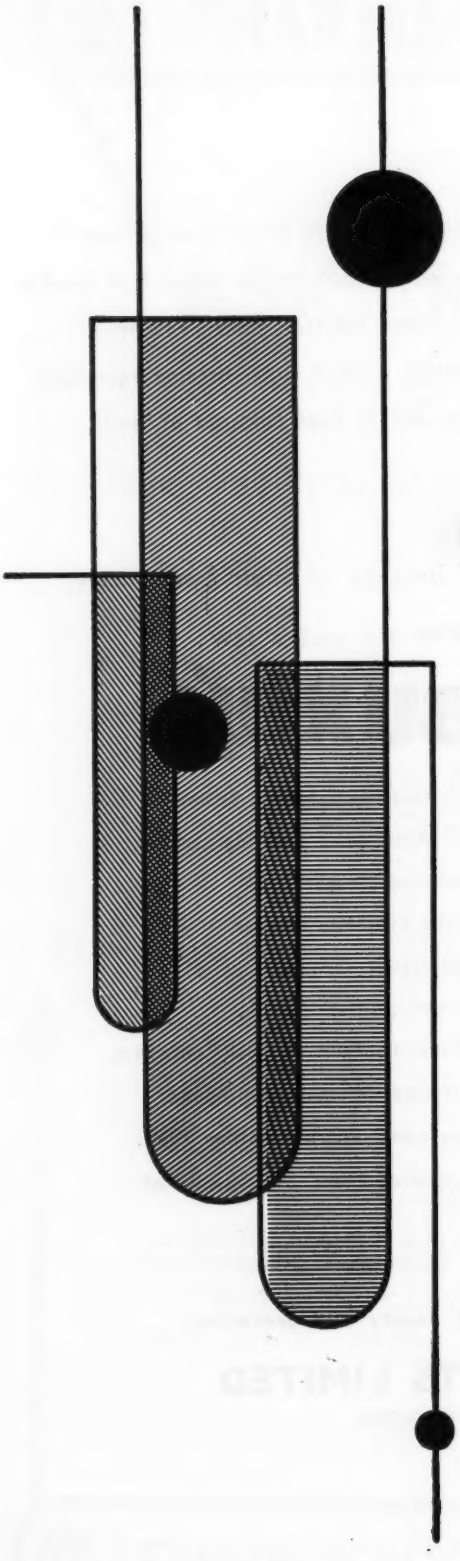

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DETERMINATION OF NITROGEN IN ORGANIC COMPOUNDS WITHOUT DISTILLATION

Part II.—Determination of Nitrogen in Nitro, Nitroso and Azo Compounds

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(Received April 13, 1960)

A Kjeldahl method for the determination of nitrogen in nitro, nitroso, and azo compounds without resorting to the usual distillation step is described. These compounds are reduced with glucose to amino compounds which yield ammonium sulphate on digestion with sulphuric acid. The ammonium sulphate is determined titrimetrically, after hypobromite oxidation. The accuracy of the method is ± 0.2 %.

The Kjeldahl method cannot be directly employed for determining nitrogen in the compounds containing N-N, NO and NO_2 groups. Clark[1] strongly recommended the Friedrick[2] method for the analysis of such compounds. According to this method, the reduction of organic compounds is brought about with hydroiodic acid previous to kjeldahlisation. Nitrogen in hydrazines, dinitrohydrozones, osazones, oximes, nitro, nitroso and azo compounds is determined with great accuracy by this method. It cannot, however, be applied to diazoketones ($R\text{-}CO\text{-}CHN_2$) which split off the whole of their elementary nitrogen with hydroiodic acid.

The use of salicylic acid[3] and sodium thiosulphate for the reduction of organic substances has also been found effective because the former compound in the presence of sulphuric acid gets converted to nitrosalicylic acid which can be reduced to amino compound by sodium thiosulphate. After digestion, the compound delivers nitrogen quantitatively as ammonia. This method has been further improved by using thiosalicylic acid which converts oxidized nitrogen to ammoniacal[4] form. Bradstreet[5] used 1-napthol-pyrogallol and obtained better results than when he used salicylic or thiosalicylic acid. The addition of phenol and zinc dust,[6] phenol, resorcinol or phloroglucinol alone,[7] zinc[8] alone, potassium persulphate and zinc dust,[9] stannous chloride[10] and sulphur[11] was also suggested for the analysis of nitro, azo and similar compounds by digestion with sulphuric acid.

Dickinson[12] reduced nitro nitrogen and nitrogen-nitrogen single bond nitrogen almost quantitatively to amino compounds by zinc dust. The reduction was carried out in non-oxidizing acid solutions using formic, acetic, phosphoric and hydrochloric acids prior to Kjeldahl digestion. Iron and aluminium were also used as reductants. This method, like various other methods mentioned above, involves an additional difficult procedure before proceeding for digestion and much time is required for making the determination. Belcher and Bhatty[13] determined nitrogen on the micro scale in nitro, nitroso and azo organic compounds by first reducing the compounds with chromous chloride and then distilling off ammonia from the digest.

Basis of the New Method

These methods, however, involved the presence of residues from reductants which would interfere in one way or the other if distillation in the

Kjeldahl method was to be eliminated. The application of glucose or sucrose as a reducing agent has also been reported [14, 15] and it has been shown that oxidized nitrogen is quantitatively converted to ammonia. Glucose [14] 1g. (or 0.5 g. sucrose[15]) was added to 0.1 g. of the sample and digestion and distillation were carried out by the usual Kjeldahl method. Such a reduction process appeared direct, easy and rapid. It would also save time from additional reduction prior to digestion. Besides, since the distillation step in the Kjeldahl method can be eliminated by a titrimetric method, the presence of interfering compounds is not called for as glucose or sucrose is got rid of during digestion of the substance with sulphuric acid. In the present investigations, glucose has been used as a reductant for nitro, nitroso and azo compounds, and the nitrogen has been determined without distillation by the modified Kjeldahl method.

The reducible form of nitrogen in a number of compounds has been determined by this method and results have been compared in Table 1. These results have been obtained by adding 2 g. of glucose to 0.1 to 0.2 g. of sample. The procedure used in Part I[16] has been slightly modified and employed in this case also. As additional carbon from glucose depletes the acid materially, the volume of sulphuric acid has been increased from 8 to 15 ml. Total time required to complete the reduction and digestion in this case is two hours. Recoveries of nitrogen from *m*-dinitrobenzene are low when this procedure is rigidly followed. However, when 3 g. of glucose and 30 ml. of sulphuric acid are used and heating is carried out below 50° C. for 1 hour and then at 360° C. for 3 hours, the recovery of nitrogen is quantitative.

TABLE I.—THE DETERMINATION OF NITRO, NITROSO AND AZO NITROGEN IN ORGANIC COMPOUNDS WITHOUT DISTILLATION.

No.	Compound	Required	Found		
1.	2,4-Dinitrophenol	15.22	15.23	15.12	15.00
2.	3,5-Dinitrobenzoic acid ..	13.20	13.40	13.37	13.24
3.	*o*-Nitrophenol ..	10.07	10.06	9.99	10.08
4.	5-Nitrosalicylic acid	7.65	7.59	7.58	7.52
5.	α-Nitroso-β-naphthol	8.09	8.11	7.95	8.00
6.	*p*-Nitroaniline	20.29	20.39	20.40	20.31
7.	*o*-Nitrobenzoic acid	8.38	8.42	8.22	8.25
8.	*p*-Nitrobenzaldehyde	9.27	9.24	9.29	9.25
9.	Azobenzene ..	15.38	15.42	15.41	15.44
10.	*p*-Nitrobenzoic acid	8.38	8.22	8.18	8.31
11.	*m*-Dinitrobenzene*	16.67	16.58	16.51	16.49

*Quantities of the reductant and sulphuric acid, and the time of reduction as well as of digestion were increased.

Discussion

It is evident from the results shown in Table 1 that an accurate determination of the reducible forms of nitrogen, such as nitro, nitroso and azo in organic compounds, is possible. The addition of glucose helps the reduction in the majority of cases, although for compounds like *p*-nitrophenylhydrazine and dinitrophenylhydrazine, satisfactory results have not been obtained.

With the development of this method it is now possible to determine almost all types of nitrogen with a single uniform procedure in which distillation has been eliminated.

Acknowledgement.—Our thanks are due to Dr. Karimullah for his helpful suggestions and for providing facilities to carry out this work.

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STUDIES ON LAVANDULA STOECHAS LINN. (LABIATAE). PART I

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(Received March 17, 1960)

A hydrocarbon most probably *n*-nonacosane, *d*-Camphor and three sterols so far unknown were isolated from the petroleum ether extract of *Lavandula stoechas* Linn. The unknown sterols were named as Lavandula Sterols A, B & C, having two, one and three oxygens in their respective formulae. Specially the one with three oxygens attracts interest as starting material for cortisone synthesis because when the plant is harvested during special time of the year it contains as much as 2.8% of this sterol C.

Introduction

As mentioned in Nadkarni's Indian Materia Medica, *Lavandula stoechas*, Linn. (Labiatae) is used under the name of Ustukhudus in Unani drugs to cure chest affections, and to relieve biliousness. The plant is also known as Dharu and Alphajana Dharu in Hindi. In antiquity Dioscorides states that this plant is called stoechas, because of growing on the Stoechades, a group of islands on the south coast of Gaul near Massilia, now called Isles d'Hyeres. In Western India, the drug is best known (though incorrectly) under the Portuguese name of Alphazema which is corrupted by natives into Alphajana.[1] In English it is known as French 'avender.

Dymock, Warden and Hooper have mentioned in Pharmacographia Indica that the plant is much used by the Muslim physicians who consider it to be cephalic, deobstruent, and carminative and prescribe it in chest affections. They also think that it assists in expelling bilious and phlegmatic humors. The author of Makhzen-el-Adwiya devotes a whole folio page to a description of its properties, and especially enlarges upon its cephalic virtues. He concludes by saying, "In short Ustukhudus is the broom of the brain; it sweeps away all phlegmatic impurities and removes obstructions, strengthening its power, expelling vain crudities and rarifying the intellect." *Lavandula stoechas* is known in Spain as Rosemero Santo, (sacred rose-mary). Its essential oil is obtained there for household use by suspending the fresh flowering stalks, flowers downwards, in closed bottles, and exposing them for some time in sun rays. A mixture of water and essential oil coellcts at the bottom, which is used as a haemostatic and for cleaning wounds.

Investigation

The preliminary investigation of the plant (stems only) was done with only 300 g. which furnished approximately 3% petroleum ether extract and 5% alcoholic extract. Specially from the petroleum ether extract, needles and a white amorphous substance could be obtained while besides an essential oil there was also present the characteristic smell of camphor. Therefore it was decided to start a fuller investigation of this plant.

The dried whole plant without roots (3.6 kg.) was exhaustively extracted with petroleum ether. Eleven extractions using in all 86.5 litres of petroleum ether (b.p. 60-70° C.) in 96 days had been necessary. On concentrating the various fractions, an amorphous greenish substance settled down which was separated from each fraction by suction on filter paper. A total of 103.03 g. of this amorphous substance equivalent to 2.8% of the plant material could be obtained while the amount of petroleum ether extract totalled up to 306.061 g., equivalent to 8.5% by weight of the plant material.

When different extracts, after removing the sparingly soluble greenish amorphous substance, were concentrated, crystals started depositing. Notwithstanding these crystals, it was subjected to adsorption analysis on alumina (Brockmann, E. Merck). The total extracts (the soluble part of petroleum ether) (83.791 g.) were dissolved in 500 ml. of petroleum ether and poured into a column of 12" length and 3-1/2"width packed with 1500 g. of Brockmann alumina (E.Merck). The ratio of the substance to adsorbant was therefore 5.5%, while the classical adsorption analysis prescribes only 2-4%, but it has been found that for larger quantities, fairly good separation could be achieved with these higher percentages. Petroleum ether, benzene, ethyl acetate, and chloroform had been used successively as eluents. One hundred and thirty fractions were collected, and in all 43.7948 g. of the substance was recovered, which means that nearly half of the starting material, namely 39.9962 g., must be still in the column. This is still under investigation. Preliminary tests made it feasible to combine the 130 fractions into four different groups, and each group was investigated separately.

Isolation of Lavandula Hydrocarbon.—Out of 5.4017 g. of the substance from Group I, a solid

substance melting at 58-61° C. was obtained by high vacuum distillation at 0.02 mm. pressure and at 208-240° C. On recrystallization this solid substance from acetone, a brittle white substance, having the constant m.p. 61-63°C., was obtained, analysis of which furnished the values C, 85.54; H, 14.59. This would agree with a straight chain hydrocarbon of the formulae: $C_{27}H_{56}$ requiring C,85.17; H,14.83; m. p. 60° C.; or $C_{28}H_{58}$ requiring C, 85.19; H, 14.81; m.p. 61.6° C.; or $C_{29}H_{60}$ requiring C, 85.20; H, 14.80; m.p. 64° C; because the original substance furnishes very nicely shaped crystals of urea inclusion compound, out of which on decomposition with water, the starting material is received back as a waxy substance floating on the surface, while urea goes in solution. The substance does not show any rotation in 1% chloroform solution. It seems to be saturated because a chloroform solution of bromine left the substance unchanged even on standing for several hours and also potassium permanganate solution was not decolorized in neutral, acidified or alkaline medium. According to the m.p. of 61-63°C., the lavandula hydrocarbon is most probably *n*-nonacosane.

Isolation of d-Camphor.—While collecting the fractions, it was noticed that the first fraction of the group II, smelled fairly strongly of camphor. Therefore the solvent from the fractions composing the group II, was removed first by distillation under reduced pressure, and afterwards by keeping the flasks in a dessicator evacuated by an oil pump, so that the small amount of remaining solvent, i.e. the petroleum ether, was removed completely. It was then thought feasible to sublime the whole camphor present in the group on a cooling finger, by introducing it by means of an adaptor. By slightly warming the bottom of the conical flask, the camphor was soon sublimed on the cooling finger in absolute purity. It was characterized by taking the melting point, which was found to be 172-173°C. (in a sealed tube) whereas the literature mentions 176°C. and 179°C. for *d*-camphor, and the rotation in 1% alcoholic solution, which was found to be +43°, while the literature mentions +44°. Besides its characteristic smell, a small crystal obtained by sublimation from group II when put in a drop of water on a slide, and observed under microscope, was found to be rotating and vibrating vigorously, which is a property of camphor.

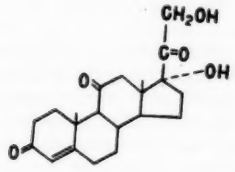
Isolation of Lavandula Sterols A and B.—Group III of fractions consisted of a yellowish oil, which crystallized on adding petroleum ether and keeping it standing. As described in the experimental part, two sterols could be isolated. The lavandula sterol A, having m.p. 204-205° C., $[\alpha]_{D}^{29} = +39.6°$ in 0.5% chloroform solution, furnished C, 80.39; H,10.08; O,9.37, fitting in the narrow range of $C_{22\text{-}23}H_{32\text{-}34}O_2$ only; and the lavandula sterol B, having the m. p. 135-136°C, $[\alpha]_{D}^{24} = -23.5°$ in 1% chloroform solution, C, 83.2; H, 11.86; O,4.94% (by difference), the molecular formula fits into the range of $C_{22}H_{36\text{-}38}O$. Both the sterols gave the positive Liebermann and digitonin tests, but they could not be identified with any known sterols. Being present in very small quantities, no further characterisation work was carried out at this stage.

Isolation of Lavandula Sterol C.—The greenish amorphous substance which settled down on concentration of the petroleum ether extract was, after purification and crystallization, proved to be a sterol, which has been named lavandula sterol C, because it could not be so far identified with any known one. In crude form, it was highly contaminated with chlorophyll and certain other impurities, wherefrom it was difficult to separate. The best method of getting the sterol in the completely white but still amorphous state was soxhlet extraction with chloroform, in which it is sparingly soluble in the cold. Its crystallisation was achieved from ethanol, when the m.p. was constant at 268-270°C., $[\alpha]_{D}^{27} = +67.2°$ in 1% tetrahydrofuran solution. Analysis report furnished the values, C, 77.42; H, 10.58; O, 12.46, which fits into the molecular formulae, $C_{26}H_{40\text{-}44}O_3$.

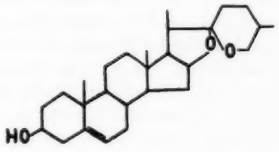
By boiling with acetic anhydride, it yielded an acetyl derivative, m.p. 278-280 °C., $[\alpha]_{D}^{26} = +65°$, the acetyl value of which was found to be 8.96, accounting for only one acetyl group. The two other oxygen atoms therefore seem to belong to other groupings. Unfortunately, the infra-red spectrogram could not be made available, therefore, the easy method of finding out if the other two oxygens are present as a lactone, ester or keto groups was not applicable. On heating with alcoholic potash, however, the substance is transferred into a difficultly soluble potassium salt of an acid formed by splitting a probable lactone ring in the molecule. The other two oxygens are therefore combined in that lactone group. This fact makes the substance considerably interesting because it might be a good starting material for the cortisone synthesis.

These hormones of the adrenal glands have achieved immense importance due to their striking efficacy in the amelioration of rheumatoid arthritis, allergy and lymphatic leukemia, and in promoting the healing of burns. The technological production of these hormones, especially of cortisone, has become an important industrial problem. The twenty steps previously encountered in the transformation of bile acids into cortisone I, could be considerably shortened by starting

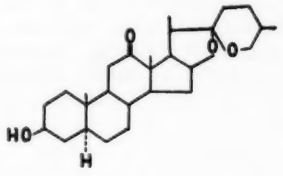
Cortisone
I



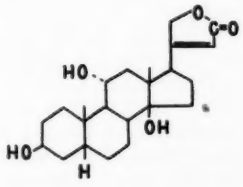
Diosgenin
II



Hecogenin
III



Sarmantogenin
IV



from more suitable compounds, e.g. diosgenin II from Mexican jam, and hecogenin III, which has been found in abundance in the juice of Agave groups of plants to which also sisal belongs. According to New Zealand Science Report of January 1959, this source is already used successfully in the Commonwealth. Finally, sarmantogenin IV, which is available from *Strophanthus sarmantosus* may be mentioned. As already pointed out lavandula sterol C is not identical with any of them. In its molecular formula it comes nearest to diosgenin, but in its behaviour towards alcoholic potash, it seems to have a lactone grouping which might be similar to that present in sarmantogenin. Its usefulness in the cortisone synthesis however will depend upon further studies on its constitution which are underway.

Experimental

Petroleum Ether Extraction.—The whole plant without roots (3.6 kg.), completely dried and crushed to small parts, was extracted exhaustively (11 times) with petroleum ether (b.p. 60°C.).

When the different extracts were concentrated to about 500 to 700 ml. under reduced pressure at 35-40°C. some of the substance settled down which was sucked off. Later experiments showed that it was a sterol which is named as lavandula sterol C. The filtrates of the extracts were individually concentrated, finally dried over phosphorus pentoxide and weighed. The weight of the sterol C was added to the filtrates to get the total weight of the individual extracts.

Different extracts were treated according to the above scheme. The results obtained are given in Table 1.

Absorption Analysis on Alumina.—Two experiments were carried out separately.

EXPERIMENT I : 30.8 g. of the first extract (including the lavandula sterol C) was taken up in 120 ml. of petroleum ether (some of the substance remained insoluble, i.e., the lavandula sterol C) and placed on top of a column of 3.5 cm. width and 40 cm. length packed with 350 g. of Brockmann's alumina (E. Merck). Petroleum ether, benzene, ethyl acetate and chloroform were used as eluents successively. Ninety-eight fractions were collected. Total eluents receieved, 15.6868 g.; remained in column, 15.132 g.; total, 30.8 g.

EXPERIMENT II : 83.701 g. of the combined 2nd to 11th extracts (without the already separated lavandula sterol C) was dissolved in 500 ml. of petroleum ether, and poured into a column of 14"

TABLE 1

Extract No.	Amount of solvent	Duration of extraction	Sparingly soluble sterol C	Total weight of extract
1	14000 ml.	75 hrs.	33.83 g.	153.07 g.
2	9500 ,,	60 days.	15.56 g.	61.49 g.
3	8500 ,,	70 hrs.	18.41 g.	35.72 g.
4	8500 ,,	72 ,,	5.32 g.	14.73 g.
5	7500 ,,	71 ,,	3.00 g.	8.34 g.
6	8000 ,,	70 ,,	10.41 g.	12.54 g.
7	8000 ,,	96 ,,	7.33 g.	8.50 g.
8	8000 ,,	87 ,,	3.40 g.	4.35 g.
9	8000 ,,	106 ,,	2.72 g.	3.58 g.
10	8000 ,,	114 ,,	1.97 g.	2.508 g.
11	8500 ,,	123 ,,	1.08 g.	1.233 g.
Total	86.5 l.	96 days	103.03 g.	306.061 g. =8.5 %.

length and 4" width, packed with 1500 g. of Brockmann's alumina (E. Merck). Petroleum ether, benzene, ethyl acetate and chloroform were used as eluents successively. One hundred and thirty fractions were collected. Total eluents received, 43.7948 g.; remained in column, 39.9962 g.; total, 83.791 g.

On the basis of physical appearance, solubility tests and microscopic examinations, the fractions have been combined into four groups as shown in Table 2.

TABLE 2

Fractions	Weight	Group	Remark
Exp. I, 1-4 Exp. II, 1-6	2.6479 g. 5.4017 g.	I	White waxy material
Exp. I, 5-25 Exp. II, 7-14	3.00225 g. 8.0131 g.	II	Yellowish oil, containing camphor
Exp. I, 26-58 Exp. II, 15-49	2.34025 g. 8.4699 g.	III	Yellow oil, crystallises on keeping longer and adding pet. ether
Exp. I, 59-98 Exp. II, 50-130	7.5964 g. 21.9101 g.	IV	Greenish yellow oil

Isolation of Hydrocarbon (Group I).—Fractions 1 to 6 from Exp. II were combined by dissolving in chloroform, and then transferred into a micro-distillation Claisen flask. The solvent was removed completely, and the white waxy substance thus obtained was distilled under high vacuum according to the following scheme:

Starting meterial	Temp. °C.	Pressure mm.	Fract.	Amount g.	Remark
5.4017 g.	120-140	0.05	I	0.258	Oily
	168-184	0.03	II	0.187	,,
	208-240	0.02	III	2.731	Solid

The fraction III was dissolved in about 100 ml. of acetone by warming on a water-bath. On cooling a brittle white substance settled down. It was sucked off on a sintered porcelain crucible and dried over phosphorus pentoxide in vacuum. Yield, 1.818 g.; m.p., 58-61°C. After the third recrystallization from acetone the m. p. was constant at 61-63°C. Found: C, 85.54; H, 14.59.

Formation of Urea Inclusion Compound.—One hundred mg. of the hydrocarbon mixed with 400 mg. of urea was heated with 10 ml. of butanol. The substances went in solution completely. On cooling to room temperature nice rod shaped crystals came out, which were sucked off. Yield, 0.194 g.; m.p., 132-134°C. The crystals were homogenous. These crystals were then dissolved in 50 ml. of hot water when urea went in solution and an oily substance started floating. On cooling it solidified like wax as the original substance. It was sucked off and dried over phosphorus pentoxide in vacuum. Yield, 0.087 g.; m.p., 59-62°C.

According to the value obtained, the range of the hydrocarbon fits in the following molecular formulae: $C_{27}H_{56}$ requires C, 85.17; H, 14.83; m. p. 60°C. $C_{28}H_{58}$ requires C, 85.19; H, 14.81; m.p. 61.6°C. $C_{29}H_{60}$ requires C, 85.20; H, 14.80 m. p. 64° C.

Isolation of d-Camphor (Group II).—Fractions 7 to 14 from Exp. II smelled like camphor. The solvents from these fractions were removed under reduced pressure. The final removal of the solvent was carried out at room temperature by keeping the flasks in a vacuum dessicator. The camphor was afterwards isolated by direct sublimation out of the receiver flask of the combined fractions by introducing a cooling finger. By slight warming the bottom of the conical flask on a water bath at 60-70°C. the camphor soon condensed on the cooler in absolute purity. Yield, 1.12 g.=0.031% of the plant material.

The sublimed substance exhibited the following properties characteristic of camphor :

(1) The substance has the characteristic smell of camphor. (2) It melts at 172-173° C. whereas *d*-camphor melts at 176°C. (179°C). The melting point was taken in a sealed tube. (3) Mixed m.p. showed no depression. (4) Optical rotation was found to be $[\alpha]_{21} = +43°$ in 1% ethanolic solution. The optical rotation of *d*-camphor as mentioned in the literature is +44°. (5) A crystal from the fraction when put in a drop of water on a slide and observed under microscope seemed to be rotating and vibrating vigorously which is a property of camphor.

Isolation of Lavandula Sterol A.—The fractions of the group III of Exp. I were combined together by dissolving in ethyl acetate. The solvent was completely removed, and the yellow oil thus

obtained was dissolved in petroleum ether by heating on a water-bath. It was then concentrated to about 5-7 ml. and kept standing at room temperature for crystallisation. After keeping it overnight, needles started coming out. The mother liquor was decanted and the crystals attached to the walls of the flask were washed twice with a little amount of petroleum ether. The clear crystals after redissolving in petroleum ether by heating on a water-bath were kept again for crystallization. Lengthy rods along with some amorphous substance came out on standing over two nights. The mother liquor was again decanted off from the rods and the amorphous substance, and after concentration left for crystallisation. Some more rods came out. The rods (lavandula sterol A) were separated from the amorphous substance mechanically (yield, 0.0236 g.) m.p.=204-205°C. (on Koflers' block).

The amorphous substance sublimed into well-defined plates, m.p.=114-115°C. on Koflers' block. Further characterisation could not be carried out due to non-availability of more of the amorphous substance.

Analysis report of lavandula sterol A shows C, 80.39; H, 10.08; O,9.37, according to these values, the molecular formula fits into the range $C_{22-23}H_{32-34}O_2$. The specific rotation was found to be $[\alpha]_D^{29}=+39.6°$.

Unfortunately all the 23 mg. of the sterol stated had been used up and therefore further investigations could not be carried out.

Isolation of Lavandula Sterol B (Group III).—The fractions of Group III from Exp. II were mixed together by dissolving in ethyl acetate (8.4699 g.). Ethyl acetate was removed completely and the remaining oil was dissolved in a few ml. of petroleum ether, and kept for crystallization. Nothing came out. Petroleum ether was then completely removed and ethyl alcohol was added when some turbidity occurred. On keeping for about a fortnight needle-shaped crystals started settling down, but in a very small amount. It was therefore cooled in a refrigerator when more of the crystals came out. It was then sucked off and dried over phosphorus pentoxide. Yield, 0.25 g.; m.p. 123-126°C.

The mother liquor was concentrated to about 10 ml. and again kept in a refrigerator. Crystals again started depositing. Yield, 0.123 g.; m.p., 124-127°C.

The I and II crops were mixed together (wt. 0.373 g.) and recrystallised from petroleum ether. Rod-shaped crystals deposited. Yield 0.16 g., m.p. 135-136°C. On three more recrystallization the m.p. was constant at 136-137°C., $[\alpha]_{24}$ −23.5°. Found: C, 83.20; H, 11.86; O, 4.94 by difference). According to the values given (the molecular formulae fits into the range: $C_{22}H_{36-38}O$. The substance gives positive Liebermann and digitonin tests.

Isolation of Lavandula Sterol C.—The substance which was sparingly soluble in petroleum ether and settled down on concentration of the petroleum ether extracts was named lavandula sterol C.

PURIFICATION.—Various experiments were carried out to purify the substance as it was contaminated with chlorophyll. Among them the soxhlet extraction with chloroform proved to be the best.

The raw substance (8.905 g.) was brought into a thimble of filter paper and soxhleted with chloroform for 6½ hours. The extracts previously dark green remained nearly colourless. The receiver was taken out and the solvent renewed. The dark green first extract was allowed to stand overnight when a lot of solid substance was observed to be floating on top of the solution. It was sucked off ad washed four times with chloroform, pressed on a porous plate and weighed. Yield, 2.274 g. The mother liquor on further concentration did not yield more of the white substance. Brought to dryness, a solid dark green mass was obtained which is still under investigation.

The substance was now completely white but amorphous. At about 224°C. shrinking starts, at 240°C. it becomes brown, and melts at 247-249° C. with decomposition. The melt solidified to a brown lacquer. No sublimation could be observed. The substance was soluble in pyridine and tetrahydrofuran; sparingly in benzene, chloroform, ether, petroleum ether and ethanol;and insoluble in water and cyclohexane. It was recrystallized from ethanol.

RECRYSTALLIZATION.—The substance (1.10 g.) was dissolved in ethanol by heating on a water-bath and after filtration was kept for crystallization. Needle-shaped crystals started coming out on standing for some time. They were sucked off on a sintered glass crucible and dried in vacuum over phosphorus pentoxide. After two further recrystallizations the melting point was constant at 268-270°C.

Found: C, 77.42; H, 10.58; O, 12.46, which fits into the following molecular formulae: $C_{26}H_{40}O_3$

requiring C,77.95; H, 10.07; O, 11.98. $C_{26}H_{42}O_3$ requiring C,77.56; H, 10.62; O, 11.86. $C_{26}H_{44}O_3$ requiring C,77.17; H, 10.96; O, 11.86.

The substance gives positive Liebermann-Burchard test for sterol. The digitonin test however was negative.

Acetyl Derivative of Lavandula Sterol C.—The substance (200 mg.) with 4 ml. of acetic anhydride and three drops of pyridine was refluxed for five hours at 140-150°C. on a glycerine bath. The substance completely dissolved in acetic anhydride on heating. Nothing came out on cooling. The mixture of acetic anhydride and acetic acid was removed under reduced pressure and water added. The outcoming substance was sucked off and dried over phosphorus pentoxide. Yield, 0.298 g.; m.p., 256-262°C.

SOLUBILITIES.—Easily soluble: tetrahydrofuran, glacial acetic acid (hot), alcohol (hot). Sparingly soluble: petroleum ether, ethyl acetate, chloroform, carbon tetrachloride, dioxane, dimethyl formamide, alcohol (cold), acetone, acetic acid. Insoluble: water. Recrystallisable: glaical acetic acid-water mixture.

The acetyl derivative was dissolved in glacial acetic acid and precipitated with water. After three repeated recrystallisations the m.p. was constant at 278-280°C., $[\alpha]_D^{26} = +65°$ in 1% tetrahydrofuran solution, the acetyl value was found to be 8.96%. $C_{26}H_{42}O_2.O.CO.CH_3$ (444) requires: 9.6% $-CO.CH_3$.

Acknowledgement.—The microanalyses reported have been carried out by A. Bernhardt, Max Planck Institut fuer Kohlenforschung, Muelheim, Ruhr, Germany.

DEVELOPMENT OF RAPID METHODS FOR THE ESTIMATION OF THE OIL CONTENT OF SINGLE COTTONSEEDS

Part IV.—Investigation of an Electrical Circuit for Measuring the Electrical Response of One Cottonseed at 1,000 Cycles/Second

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In an effort to develop further the electrical method or non-destructive estimation of oil in a single cottonseed, an extension circuit has been devised for increasing the sensitivity of the Marconi universal bridge, and its behaviour as well as the characteristics of the detector arrangement are studied for various percentages of imbalance. In this way, a sensitivity of almost the required order is attained, and some observations have been made with one to five kernels of M4 and L.S.S. cottonseeds. The effect of humidity is observed and the variation of dielectricconstant K of the cake with relative humidity has been measured. At theoretical calculation of the value of K for optimum discrimination between oil and cake is made, and the best value of relative humidity is deduced.

1. Introduction

In the previous communication on non-destructive techniques of oil estimation, a preliminary examination of the electrical methods was made,[1] and a tentative formula was developed for the estimation of oil in gramme-quantities of cottonseed kernels by capacitance measurements and by a combination of capacitance and conductance measurements. With the normal sensitivity obtainable with the Marconi universal bridge, (Type TF 868 A), several grammes of seeds were found to be necessary for a reliable estimation, and it is the purpose of the present investigation to study the possibilities of a simple extension of this technique to single cottonseeds.

For this, three factors need to be investigated: (1) the relation between the number of seeds and their capacity and conductance response, (2) the possibility of increasing the sensitivity of the bridge a hundred fold so as to obtain reliable estimations of oil with a single seed, and (3) the influence of atmospheric humidity on the electrical response. All these aspects are studied in the present paper, and the developments include a null-point capacitance balancing device and investigation of the optimum relative humidity and water content of the cottonseeds for the best results from this electrical method.

2. Preliminary Measurements with 5, 10, 15, etc. Cottonseeds

These measurements were made with the condenser described previously[1] (Fig. 1a), using weighed lots of five kernels, which were successively added to the insulated container between the condenser plates which were nearly 8 mm. apart. The weight of the kernels in the container was recorded together with the mean of three corresponding capacity and phase angle readings. The capacity readings were estimated to be reproducible to within 0.03 $\mu\mu$F and a plot of ΔC against weight of the kernels (in the container) is shown in (Fig. 1b) (hollow circles) for a temperature of 29°C. and a relative humidity of $65 \pm 5\%$. It is seen that a smooth graph, linear at the origin, can be drawn through the experimental points, none of which deviates the graph by more than the estimated error of 0.03$\mu\mu$F. The graph as a whole is non-linear and rises steeply when the number of kernels exceeds 15 (wt. $\backsimeq$0.6 g.), while the portion for less than 15 kernels can be considered as approximately linear.

The whole curve can be fitted by the equation

$$\Delta C = (0.14\ m + 0.21\ m^2)\ \mu\mu\ F, \qquad 1(a)$$

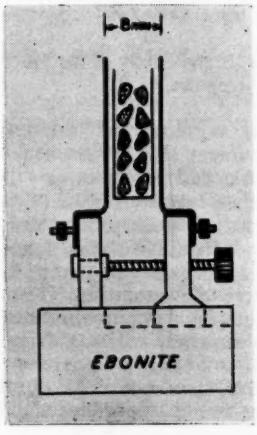

Fig. 1.—Diagrammatic sketch of measuring condenser with adjustable square plates (2.5 cm. side) and insulated container for holding the cottonseed kernels.

where m is the mass in g., and the non-linearity of the response was at first thought to be due to the weakening of the electrical field near the bottom edges of the condenser plates. This idea was examined by repeating the above measurements (i) with the bottom of the insulated container 6 mm. above the lower edge of the plates and (ii) with the same container placed between larger plates (4.5 cm. side as against 2.5 cm. previously). The results of (i) are shown by crosses in Fig. 1(b), and they exhibit no significant departure from the previous curve, while the results of (ii) are plotted in Fig. 1(c) and can be fitted by the equation

$$\Delta C=(0.19\ m + 0.19\ m^2)\ \mu\mu\ F \qquad (1b)$$

which indicates fair agreement with equation 1(a), thus showing that only a small part of the parabolic component can be attributed to the edge-effect. From the mean of the two equations 1(a) and 1(b), viz. $\Delta C=m\ (0.17+0.20\ m)\ \mu\mu$ F, it follows that (a) for 1 to 2 g. of kernel, ΔC is of the order of 0.5 $\mu\mu$ F/g. , in agreement with the previously reported measurements, and (b) the capacity response for a small number of kernels will be nearly constant at about $(0.19\pm0.02)\ \mu\mu$F per g., corresponding to a little less than 0.01 $\mu\mu$F per kernel of 40 mg. each.

This is of the correct order of magnitude predicted by the previously derived[1] theoretical equation for kernels of mean dia. D, viz.

$$\Delta C=(1-1/k)+\frac{\text{Volume of sample}}{4\pi t^2} \div\{1-\alpha(1-1/K)\ D/t\}$$

$$\simeq(1-1/K)\left\{1+\frac{\alpha D}{t}\right\}\times\frac{\text{Volume of sample}}{4\pi t^2} \qquad (1c)$$

where $\alpha\simeq 0.9$ and t is distance between the conductor plates. For measuring such extremely small capacity changes, the extension circuit, described below, was set up.

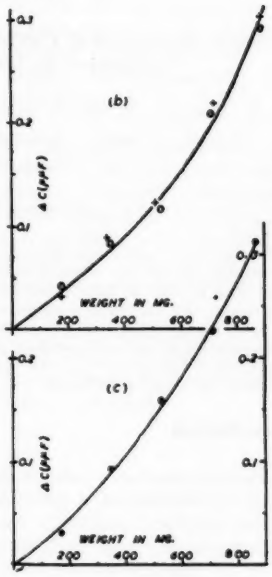

Fig. 1(b).–Measured response of 5 to 25 kernels, plotted as capacitance change against weight of kernels in the container: hollow circles, first set; crosses, second set with bottom container 6 mm. above the lower edge of the plates.

Fig. 1 (c).-Capacitance response for 5 to 25 kernels measured in a condenser with larger plates (4.5cm. side).

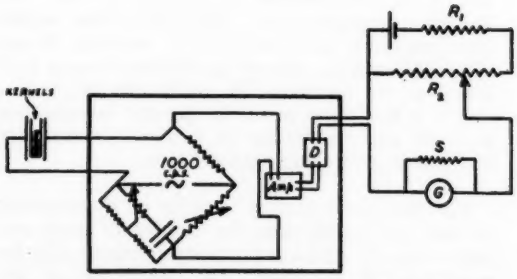

Fig. 2.—Sketch of extension circuit increasing the sensitiviy of the capacitance bridge.

3. The Extension Circuit and Study of the Detector Response

The extension circuit replaces the side-zero panel meter of the instrument and is sketched in Fig. 2, where the blocked portion is the normal circuitry inside the Marconi bridge (Type TF 868A.) The 6-volt battery and series resistances R_1 (250 ohms) and R_2 (200 ohms) supply the small steady current necessary to keep the deflection of the sensitive glavanometer 'G' on the scale, and 'S' is a shunt for damping unwanted oscillations of the glavanometer. The galvanometer used had a sensitivity of about 0.3 microamp. per division with an internal resistance of 208 ohms, and the above circuit was found to produce stable deflections of the pointer, which were nearly twelve times the corresponding deflections on the bridge panel meter.

The procedure adopted was first to balance the bridge with the panel meter, and then to insert the jack plug, which (while disconnecting the panel meter) connects the extension circuit. R_2 was then adjusted to bring the galvanometer deflection near the middle of the scale and the bridge could then be balanced more accurately with the help of this galvanometer. Since the capacity balance is indicated by a "stationary" value of deflection of the galvanometer, it is clear that the limits of sensitivity will be determined

by the response characteristics of the detector circuit, which rectifies the alternating out-of-balance voltages from the bridge proper and then feeds them to the indicating circuit. In the foregoing experiment it has been seen that when the distance between the plate is 8 mm. (giving a capacity of 2 to 3 $\mu\mu$F), the capacity change is nearly 0.01 $\mu\mu$ F per kernel, up to a total of 10 kernels. If the circuit is to measure the oil in a sample composed of only a few kernels, then it must give a clear indication for as small a capacity change as 0.01 $\mu\mu$ F or even less, if possible. Therefore it was considered desirable first to study the response of the set-up of Fig. 2 to capacity changes in the range of 0.01 to 0.2 $\mu\mu$F.

This was done by connecting the measuring condenser (Fig. 1(a))of capacity $C_\circ$, at about 3 $\mu\mu$F, in series with a variable condenser C of capacity 45 to 450 $\mu\mu$F, using stiff copper connections to avoid stray changes of capacity. By varying the large condenser, very small calculated changes of capacity in system can be produced, because the total capacity C_t is given by

$$C_t = 1/\left(\frac{1}{C_\circ} + \frac{1}{C}\right) = C_\circ/\left(1 + \frac{C_\circ}{C}\right)$$
$$= C_\circ\left\{1 - \frac{C_\circ}{C} + \left(\frac{C_\circ}{C}\right)^2 \ldots\right\}$$
$$= C_\circ - \frac{C_\circ^2}{C}\left\{1 - \frac{C_\circ}{C} + \ldots\right\}$$

Since $C_\circ/C < \frac{1}{15}$, we get, to a sufficient accuracy,

$$\Delta C_t = (C_t)_1 - (C_t)_2 = C_\circ^2/C_1^2 - C_\circ^2/C_2^2$$
$$= C_\circ^2\,\Delta\,(1/C) \simeq C_\circ\,(C_\circ/C)\,(\Delta C/C) \qquad (2)$$

With the capacity C at 450 $\mu\mu$F, the bridge was balanced with the help of the extension circuit, which gave an estimated accuracy of $\pm$ 0.03 $\mu\mu$F i.e. $\pm$ 1% in the bridge dial setting. The galvanometer deflection was then noted for different settings of C (from which the values of ΔC were calculated as above) and the mean deflections for two sets of observations were plotted against ΔC_t (lowest graph of Fig. 3). The curve is seen to be parabolic and is well represented by the equation.

$$\text{deflection} = 280 \times (\Delta C_t)^2, \qquad (3)$$

which shows that the sensitivity as given by the slope, i.e. 560 ΔC_t divisions $\mu\mu$F, is proportional to ΔC_t and is very poor for $\Delta C_t < 0.05$ $\mu\mu$F.

The bridge dial setting was now increased a very little bit, the imbalance amounting to about 0.15 $\mu\mu$ F (i.e. 4%), and the galvanometer zero was set by adjusting the resistance R_2. Starting

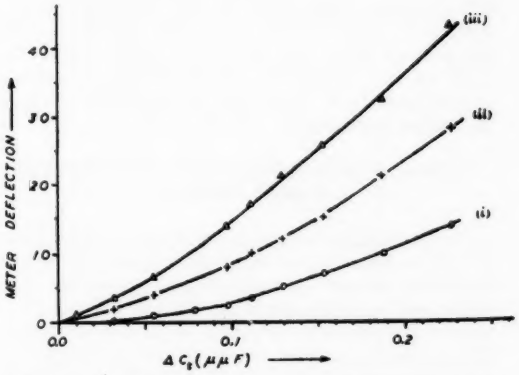

Fig. 3.—Graphs showing the measured response of the circuit arrangement of Fig. (2) for a given change ΔCt in the capacitance of the measuring condenser, starting from (i) exact balance, lowest curve, (ii) + 4% imbalance, middle curve (iii) + 7% imbalance, top curve.

with this new zero, the deflections were noted for several setting of the condenser, C, and the middle graph of Fig. 3 shows the new dependence of the deflection on the capacity change ΔC_t. The equation of this graph is approximately

$$52\,\Delta C_t + 300\,(\Delta C_t)^2.$$

whence the sensitivity is now

$$52 + 600\,\Delta C_t, \qquad 4(a)$$

which is 65 divisions / $\mu\mu$F for $\Delta C_t = 0.02$ $\mu\mu$F and 112 for $\Delta C_t = 0.1$ $\mu\mu$F. Relatively more uniform sensitivity can be obtained by increasing the off-balance setting, e.g. for an unbalance of about 7% (upper curve of Fig. 3). the sensitivity is

$$100 + 700 \times \Delta C_t\ , \qquad 4(b)$$

which varies between 100 and 170 divisions per $\mu\mu$F in the range of 0.00 to 0.10 $\mu\mu$F, so that a change of 0.01 $\mu\mu$ F would be clearly indicated as a galvanometer deflection of 1 division.

It would appear from the above measurements and discussion that the sensitivity can be increased as much as desired by increasing the amount of the imbalance. However, two factors militate against this; firstly, the automatic gain control in the amplifier becomes effective when the imbalance exceeds about 15% and secondly small fluctuations in the output from the amplifier are increased proportionally to the out-of-balance current, thus producing increased instability of the galvanometer indications, which cancels out

the advantage gained by the increased deflections. For both these reasons, the optimum setting with the present setup appears to be in the neighbourhood of 10% imbalance, which would correspond to a sensitivity of about one galvanometer division for capacitance change of 0.005 $\mu\mu$ F.

4. Some Typical Results and the Importance of Relative Humidity

Several series of measurements on small numbers of seeds were made with the extension circuit described above, using the settings of the pre-calibrated variable condenser (45 to 450 $\mu\mu$ F) to obtain a null-point position as described below. The variable condenser was set at its highest setting, thus giving the lowest capacity of the series system, which was then balanced approximately with the bridge dial, after which the extension circuit was plugged in, the bridge dial set about 10% out of balance, and the rheostats adjusted to bring the (extension) galvanometer reading near the middle of the scale. The arrangement was then ready for use in estimating the capacity changes produced by putting small numbers of cottonseed kernels in the insulated container, already placed between the plates of the fixed condenser shown in Fig. 1 (a).

One weighed kernel was dropped into the container and the variable condenser adjusted to restore the initial galvanometer position, the new reading of the condenser scale being noted. Another weighed kernel was dropped in, and the initial galvanometer position again restored, and so on, until half a dozen seeds had been put into the container, which was finally weighed as a check. The total capacitance increase for each stage was calculated from equation (2), and was of the order of $0.08 \times (\Delta C \; C)$ $\mu\mu$ F.

Some typical results obtained with M 4 & L.S.S. seeds kept at the prevailing relative humidity of 85% are shown in Fig. 4 (a) where the total change in capacitance is plotted against the total weight of kernels in the container. Although the graphs still show a certain degree of non-linearity, of about the same order as that of the curves of Fig. 1, it is seen that the average scatter of the points about the curve is only 0.002 $\mu\mu$ F, i.e. about 5% of the plotted capacitance change, ΔC_t for 5 kernels. This indicates that an accuracy of this order or a little better is all that is attainable using 1 to 5 kernels in the present setup. This serves to point up the need for even more sensitive measuring apparatus. The curve for M 4 kernels was repeated at a time when the atmospheric humidity was 75%, i.e. 10% lower than for Fig. 4 (a) and this time values of the capacitance response about 15% lower were observed (Fig. 4 b). This indicated the need for investigation of the influence of atmospheric humidity and water-content of the kernels on their effective dielectric constant. Experiments in this direction were therefore undertaken using cottonseed cake in order to eliminate the effect of the oil.

5. The Influence of Water-Content on the Dielectric Constant of Cottonseed Cakes

These experiments were carried using 6 cm. diameter pressed discs (thickness about 5 mm.) of cottonseed cake prepared in a small laboratory hydraulic press in the following way. A commercially available sample of cottonseed cake was washed with petrol, freed from bits of shell by sieving,

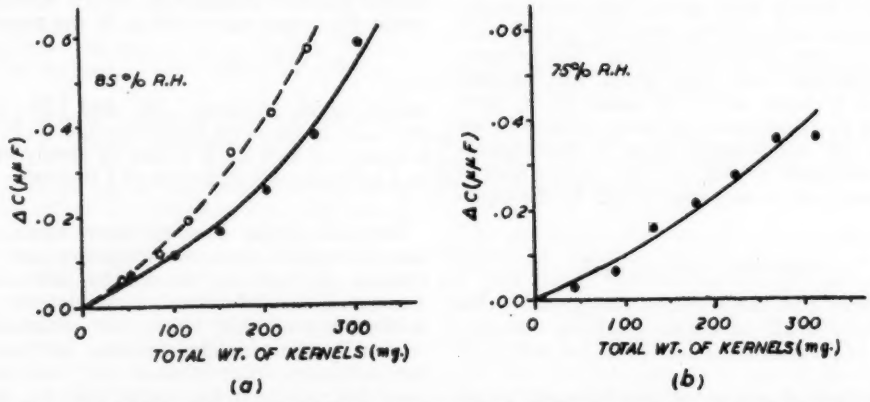

Fig. 4.—Typical results obtained with 1 to 5 cottonseeds, using the set up of Fig. 2 with 10% imbalance for (a) M4 and L.S.S. kernels at 85% atmospheric humidity; and (b) M4 kernels at 75% humidity.

TABLE 1.—DIELECTRIC CONSTANT OF PRESSED COTTONSEED CAKE MEASURED AT DIFFERENT RELATIVE HUMIDITIES.

Rel. humidity	100%	83%	54%	0%
Dielectric constant (K)	140±15	19.4±2	8.5±2	3.2±.3
1/K	.007$_1$	.052	.118	.313
Water content (%)	12.9	8.8	4.4	0.0

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The dielectric constant of the sample was first measured at the prevailing humidity, which was about 80%. The sample was then saturated with water vapour for about 20 hours, after which its dielectric constant was again measured. It was then put through one hydration→dehydration half cycle, and the corresponding values of 'K' for each value of the relative humidity were measured and are recorded in Table 1. A more accurate measure of wetness of the sample is its percentage water-content and this has also been recorded in every case by comparison with the mass of the completely dry sample, dried in a $CaCl_2$ desiccator for about 24 hours. (The samples were maintained at each value of relative humidity for this period in order to attain a satisfactory approach to equilibrium). It is seen that the dielectric constant increases rapidly with water constant, especially above 5% water, the maximum recorded value being about 140 for the fully saturated cake. Figures 5(a) and 5(b) show the graphs for 1/K against relative humidity and % water content, respectively.

In view of the above wide variation in the value of K, it is desirable to investigate mathematically its influence on the ability of a capacitance measurement on a kernel to give an accurate estimate of

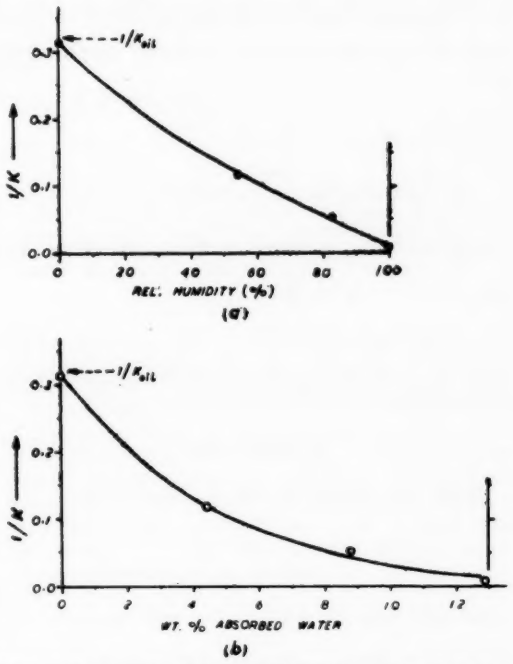

Fig. 5.—Measured variation of dielectric constant K of a sample of cleaned cottonseed cake, plotted as (a) 1/K against relative humidity and (b) 1/K against % water absorbed by the cake.

the oil content. If the kernel contains x_o parts of oil of dielectric constant K_o and $(1-x_o)$ parts of proteinous matter of dielectric constant K_p, then the dielectric constant K_m of a homogenous mixture of the two should, according to the equation of Lichtenecker,[2] be given by

$$\ln K_m = x_o \ln K_o + (1-x_o) \ln K \qquad (5)$$

whence for a small change δx_o in the value of x_o,

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Now, the capacity response due to the insertion of a single kernel is, from equation 1(c), approximately proportional to $(1 - 1/K)$ so that the change in this response is proportional to $\delta(1/K_m) \div \delta x_o$, i.e. to

$$-(4 K_m/K_m^2) \div \delta x_o = -(\delta K_m/K_m) \div (K_m \delta x_o)$$
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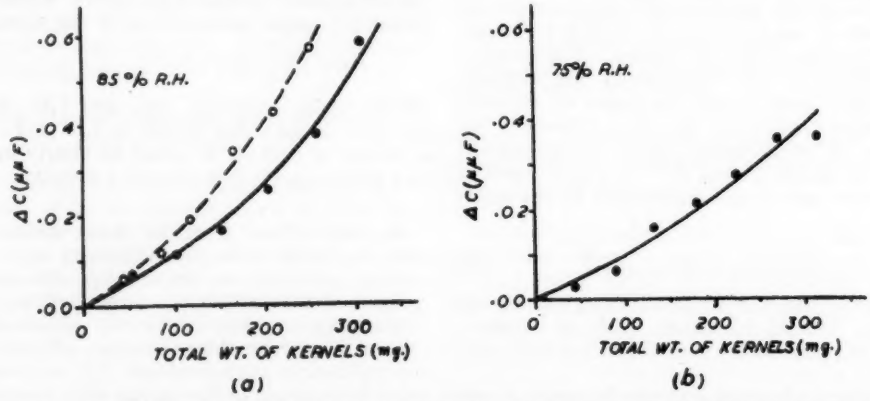

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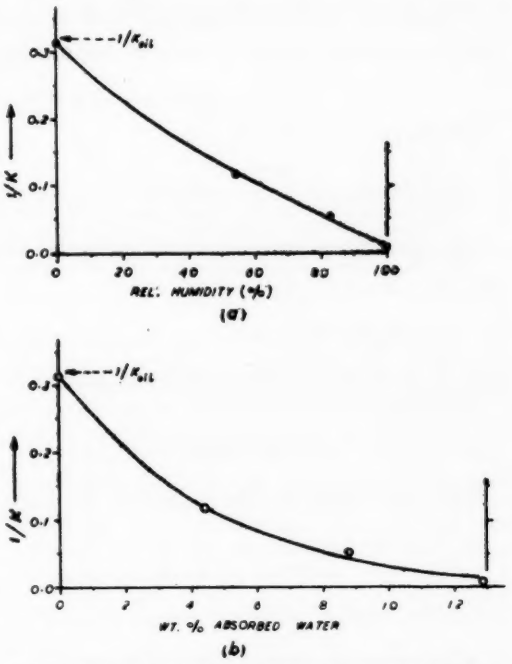

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$$K_m = K_o^{1/3} K_p^{2/3} = K_o(K_p/K_o)^{2/3}$$

and therefore the sensitivity becomes proportional to

$$\left\{\ln(K_p/K_o)\right\} \div (K_p/K_o)^{2/3}$$

$$= \frac{3}{2}\left\{\ln(K_p/K_o)^{2/3}\right\} \div (K_p/K_o)^{2/3}$$

which is readily shown to be a maximum for $(K_p/K_o)^{2/3}=e$, i.e. for

$$K_p/K_o = e^{3/2} = 4.5 \qquad (7)$$

Since K_o is about 3.1, this yields an optimum value of 14 for K_p, i.e. 0.07 for $1/K_p$.

6. Conclusion

From the graphs of Fig. 5, this value of K_p is seen to correspond to a water content of about 5% to 6% on the weight of cake and a relative humidity of nearly 70% to 80%. This corresponds to the conditions prevailing in the measurements plotted in Fig. 4, the results of which can therefore be taken as representative of the optimum performance of the present set up for single seeds, namely an accuracy of the order of 5% or perhaps a little better. While further efforts are being directed towards improving this accuracy, it should be noted that this figure is based on the experimental scatter of $\pm$ 0.002 $\mu\mu$ F, which is the smallest value obtainable at present with the best commercially available capacitance bridges.

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ON THE RADIO-FREQUENCY SYSTEM OF A 30 MEV MICROTRON. PART II*

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The main advantage of a microtron over other types of electron accelerators are indicated, and a general description of a 30 Mev microtron is given. Consideration of the requirements of a cavity resonator from the point of view of microtron dynamics leads to the choice of a conical line resonator with flattened cones and cylindrical surface. The resonant frequency of the resonator is calculated by the method of perturbation. The resonator was designed and constructed in the laboratory and its electrical properties measured. The results are found to be satisfactory.

Introduction

The conventional cyclotron is quite unsuitable for accelerating electrons to more than a few tens of kev., because the relativistic increase of mass causes a departure from synchronism between orbit period and the period of the radio-frequency supply. The synchrotron and the betatron can be used to produce high energy electrons for studying the charge distribution in nuclei through the scattering of fast electrons by these nuclei. These machines are relatively complicated and tend to be unreliable in operation; also, the intensity of the beam from such machines is rather low for accurate scattering experiments, due to the difficulty of extracting the beam.

The linear accelerator is very suitable for getting an intense beam of fast electrons, and with the development of pulsed microwave oscillators and amplifiers, capable of delivering megawatts of pulsed power at centimetre wavelengths, it is now possible to visualize linear electron accelerators using wave-guide techniques to provide energies approaching 1 Mev. per foot of accelerator.

Veksler[1] pointed out that when the peak voltage across the accelerating gap has certain discrete values, corresponding generally to integral multiples of the electron restmass, then a resonant acceleration which is in some ways analogous to that occurring in the cyclotron can take place without the complication of frequency modulation or varying magnetic fields. This principle is used in the microtron or the electron cyclotron which is capable of producing electrons of constant and known energy with ease of extraction of the beam.

A 4.5 Mev. microtron was built at University College, London. This microtron was found to be remarkably stable in operation and the beam was brought out of the machine by very simple means.[2] This machine uses a relatively low, steady and uniform magnetic field, and thus the construction of the magnet is simplified and there is little trouble from field inhomogenities. The R.F. system is far simpler and requires less precision machining than that in a typical linear accelerator. With the confidence gained during the operation of this small machine, the design and construction of a 30 Mev. microtron was undertaken. The author was associated with the development of the radio-frequency system of the machine.

* A major part of this work was carried out during the author's stay in the University College, London.

1. General Description of the 30 Mev. Microtron

The principle of magnetic resonance on which the operation of microtron rests has been discussed in great detail by various authors [3, 4, 5] since its introduction by Veksler in 1945. The microwave resonant cavity is placed near the edge of a steady uniform magnetic field, with its axis of symmetry perpendicular to the direction of the magnetic field. The resonator is excited so that the peak voltage across the lips is slightly larger than the voltage corresponding to the rest mass of the electron. Electrons are emitted from one of the lips by field emission. The values of the magnetic field and the operation frequency of the cavity are adjusted so that the electrons which emerge from the hole with a total energy of two rest masses require a time corresponding to two cycles of the radio-frequency field to complete their first orbit. They will then make the next transit of the cavity at the appropriate phase for each electron to gain one additional rest mass of energy. Since the time needed for an electron to complete an orbit in the magnetic field is directly proportional to its total energy, the second orbit corresponds to a time interval of three cycles of radio-frequency field and the electrons under consideration once more arrive at the cavity at the correct phase for each to receive one additional rest mass of energy. In this manner the electron is subject to continued accleration moving in orbits of increasing radii, all contangential to the axis of the resonator. Thus after a transit of the accelerating field, the total energy

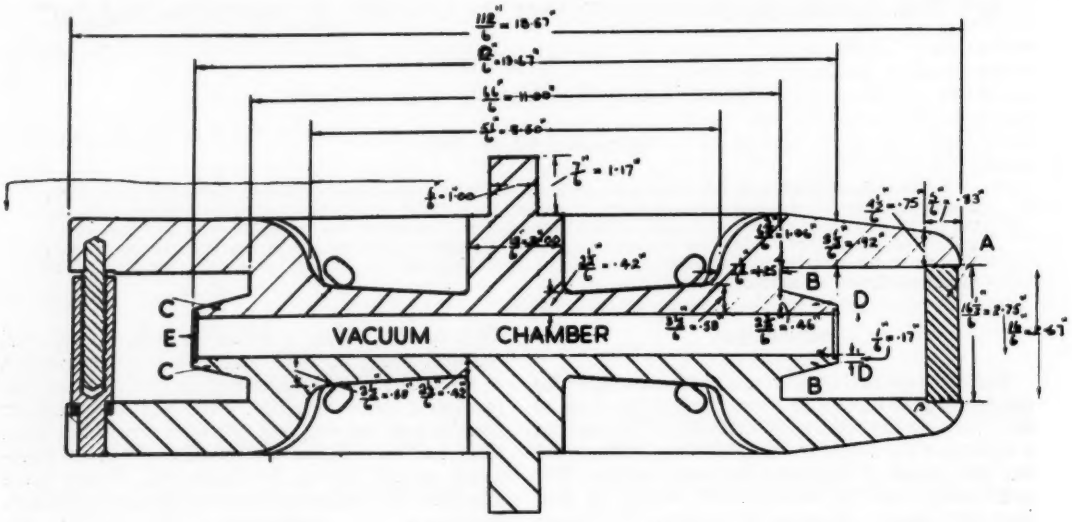

Fig. 1

of an electron is (n+1) rest masses.

The magnet has a pole diameter large enough to accommodate at least 50 orbits, with a possible upper limit of 60 orbit, in the region of uniform field. Accordingly a pole diameter of 80″ has been chosen. The air gap is 5″ so as to accommodate the larger diamension of a standard 3″× 1.5″ s-band waveguide with sufficient oom for possible auxiliary apparatus above and below the resonator. The magnetic flux density in the air gap is 1070 gauss at a wavelength of 10 cm., but the magnet is designed to provide up to 1,800 gauss to allow for possible future reduction of the wavelength. The general shape of the magnet is shown in section in Fig. 1.

Power is supplied to the magnet by a stabilized six-phase grid-controlled mercury vapour rectifier. The current supplied to the magnet is held constant to within 0.1% during normal fluctuations of the supply voltage and the increasing resistance of the magnet coils (as their temperature increases under load). At full excitation the magnet requires about 8 amps for 1070 gauss and 18 amps for 1600 gauss. The field distribution in a one-sixth scale model magnet was measured by the electron resonance method.

The vacuum system is formed by an aluminium ring sealed to the upper and lower poles of the magnet by means of "0" ring seals. Suitable ports are provided in the ring for the entry of the R. F. waveguide and for the extraction mechanism.

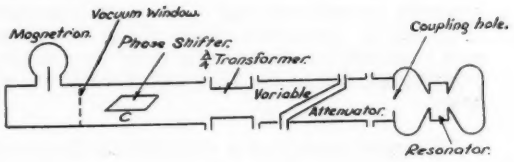

Fig. 2.—R. F. transmission system.

The radio-frequency system shown in diagram in Fig. 2 consists mainly of three parts: (*a*) the R.F. source, (*b*) the R.F. transmission system, and (c) the cavity resonator.

The source of radio frequency power is a type BM 735 pulsed magnetron capable of a rated peak output of 2000 kw. operating at 3000 MC/S. The modulator is of conventional design with an ignitron switch, and provides 3-microsecond pulses at a repetition rate of 100 pulses per second. The magnetron is coupled directly to the transmission system, the coupling being pre-adjusted during manufacture. The electrical length of the transmission line can be varied by means of a tapered dielectric line lengthener operated through a Wilson seal.

A full discussion of the R.F. transmission system and the conditions to be fulfilled for stabilization of the magnetron with resonant load has been published earlier in Part I of this communication[6] while the design and construction of the cavity resonator are described below in the next section.

2. The Cavity Resonator

From the point of view of microtron dynamics, the two major factors of the resonator are its gap parameter and outside dimensions :—

(i) The gap length must be smaller than a critical value depending on the operating mode, if electrons from a field emission source are to be accepted efficiently into phase stable orbits.

(ii) The outside dimensions of the resonator must be such that the first orbit clears the resonator.

Electrically, the resonator should have a high shunt resistance in order to minimise the power required to develop the required electric accelerating field. This requirement is in general not compatible with condition (i) outlined above. The Q-factor of the resonator should be also as high as possible to minimise the power losses in the walls.

The Gap Parameter.—Apart from the first orbit the condition necessary for resonant acceleration in the microtron is that the increase in energy obtained by an electron at each passage across the gap of the resonator shall be such as to make the electron take a whole number of periods longer for each successive orbit. Many different modes are possible. It has been shown by Heymann[2] that for a 10-cm. radio-frequency field, the best choice is the mode in which the electron gains energy corresponding to one rest mass per transit through the resonator, which therefore has a peak voltage slightly above 0.5 million volts across its lips, and the magnetic field is so adjusted that the time for each successive orbit increases by one period of the radio-frequency field. Under these conditions the gap parameter must be less than 0.81 cm. in length if phase stable operation of the microtron is desired. A gap length of 0.762 cm. was chosen for the final cavity.

The outside dimensions of the resonator and the diameter and length of the cylindrical holes through the cones are determined from consideration of clearance of the first orbit. From the electrical point of view, the hole diameter should be as small as possible and its length should be large enough to prevent radiation. But from the point of view of clearance of the beam, the hole must not be less than a minimum in its diameter and must not be greater than a maximum in its length. Hence compromises have to be made in selecting the size of the holes, and values of 0.375″ in diameter and 0.25″ in length are found to satisfy all the conditions reasonably well.

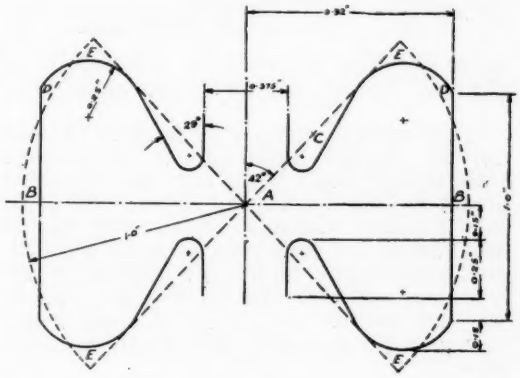

Fig. 3

Design Considerations of Shape.—Having fixed the gap length, outside size and hole parameters, the inside shape of the cavity was determined to get the highest Q and shunt resistance with the simplest possible mathematical considerations. The properties of conducting enclosures of various shapes were examined. The conical line resonator with flattened cones and cylindrical surface as shown in Fig. 3 (solid line) was found to be the most suitable for the purpose. The dotted line shows the form of the ideal conical line resonator whose electrical characteristics can be exactly calculated. The ideal cavity was modified in five regions A, B, C, D, and E to make the actual cavtiy.

The change of resonant frequency due to these modifications was calculated by the method of perturbation given by Cunlife and Mathias.[7] In actual operation the resonator had to be coupled to the waveguide conveying power from the magnetron. The coupling hole would decrease the resonant frequency of the resonator by an amount depending on its size. The change of resonant frequency due to the coupling hole was determined experimentally as it could not be calculated by simple mathematical methods.

3. Theoretical Calculation of Resonant Frequency

The resonant frequency, Q-factor and shunt resistance of cavity formed by two cones inside a perfectly conducting sphere, the apices of the cones being insulated respectively are given by

$$f = \frac{1}{4a\sqrt{\mu\varepsilon}} \qquad (1)$$

$$Q = \frac{30\pi^2}{R} \times \frac{\ln(\cot\theta_0/2)}{\ln(\cot\theta_0/2) + 0.825\ \mathrm{cosec}\,\theta_0} \qquad (2)$$

$$R = \frac{14400\pi}{R} \times \frac{[\ln(\cot\theta_o/2)]^2}{[\ln(\cot\theta_o/2)+0.825\ \mathrm{cosec}\ \theta_o]} \quad (3)$$

where a=radius of sphere, θ_o = semi-angle of the cone, $R = 2.61 \times 10^{-7} f$ for pure copper.

In our cavity the apices of the cones are flattened and the spherical surface around the meridian plane at right angles to the cone axis is pushed inward to form a cylindrical surface. We shall treat this cavity mathematically in a way that the deformations are considered as small perturbations which change the resonant frequency slightly but have negligible effects on the field quantities. We shall calculate the change of resonant frequency by the methods of perturbation discussed below.

Methods of Perturbation.—For and two vectors A and B we have

$$\int_v B.\,\mathrm{curl}\,A\,dv = \int A.\,\mathrm{Curl}\,B\,dv, \quad \text{if } A\times ds \text{ or } B\times ds = o \quad (4)$$

Now let $e=e_o$, $k=k_o$ and $h=h_o$ before perturbation and $e=e_o+e_1$, $k=k_o+k_1$ and $h=h_o+h_1$ after pertubation. In general the incremental electric and magnetic fields e_1 and h_1 inside the perturbed volume will differ from those outside it in the cavity. Let us put $e_1=e_1'$ and $h_1=h_1'$ inside the perturbed volume which is very small in comparison with the total volume of the cavity.

By Maxwell's equation we have

$$\mathrm{Curl}\ e=kh;\ \mathrm{Curl}\ h=ke;\ k^2=w^2\mu\varepsilon \quad (5)$$

In equation (4) first let $B=h_o$ and $A=e_o+e_1$ and integrate over the total volume v of the perturbed cavity; and secondly let $B=h_o+h_1$ and $A=e_o$ and integrate over $(v-\Delta)=v_o$

Thus we get $(k_o+k_1)\int_{v_o} h_o\,(h_o+h_1)\,dv$

$$+(k_o+k_1)\int_{\nabla} h_o\,(h_o+h'_1)\,dv$$

$$= k_o\int e_o(e_o+e_1)dv + k_o\int_{\nabla} e_o\,(e_o+e_1')\,dv$$

and $k_o\int_{v_o} h_o\,(h_o+h_1)\,dv = (k_o+k_1)\int_{v_o} e^{\circ}\,(e_o+e_1)\,dv$

Substracting and neglecting higher than the first order terms, we get

$$\frac{k_1}{k_o} = \frac{\int_{\nabla} e_o^2\,dv - \int_{\nabla} h_o^2\,dv}{\int_{\nabla} e_o^2\,dv + \int_{v_o} h_o^2\,dv} \quad (6)$$

Another formula given by Cunliffe and Mathias[7] is as follows:

$$\frac{k_1}{k_o} = \sqrt{\left\{\frac{1+\int \nabla e_o^2\,dv \Big/ \int_{v_o} e_o^2 dv}{1+\int h_o^2\,dv \Big/ \int_{v_o} dv}\right\}} - 1 \quad (7)$$

The actual cavity has the form shown by the solid line in Fig. 3, while the dotted line shows the form of the theoretical conical line resonator, with $\theta_o=42°$ and radius equal to 2.54 cm., the resonant frequency f being 2943 megacycles per second. The theoretical cavity is perturbed in five regions, A, B, C, D and E to form the actual cavity. From the dimension of the cavity and the above deformations, the changes of frequency due to the perturbed regions are calculated from formulae 6 and 7, and the mean is taken. The results are tabulated in Table 1.

TABLE 1.—CHANGE OF FREQUENCY DUE TO PERTURBATION IN REGIONS A,B,C,D,E.

Region	Change of frequency by formula (6) (Mc/Sec.)	Change of frequency by formula (7) (Mc/Sec.)	Mean change (Mc/Sec.)
A ..	207.70	203.30	205.50
B ..	21.40	21.00	21.20
C ..	−54.76	−57.94	−56.35
D ..	−68.29	−68.10	−68.20
E ..	35.60	35.00	35.30

The calculated resonant frequency of the final form of cavity is 3076.36 Mc/Sec.

4. Construction and Operation of the Cavity

The body of the resonator was machined from copper, while the discs with conical poles were machined from brass. Inside surfaces of the discs and main cylindrical block were highly polished and coated with silver by evaporation in vacuum. The resonator was water-cooled through a series of holes in the body. It was decided to make the resonant frequency of the resonator nearly equal to that of the magnetron. This was done in the following way. As the magnitude of the decrease of reso-

nant frequency of the resonator due to the coupling hole in it was not known, an experimental cavity was first constructed with a theoretical resonant frequency higher than the required value. The resonant frequency of this experimental cavity with a small coupling hole was determined experimentally. The difference between the measured value and the theoretical one was assumed to be the effect of the coupling hole. The resonant frequency was then made equal to the required value by altering the inside dimension of the cavity by an amount estimated by the method of perturbation. It might be mentioned in this connection that the magnetron was capable of tuning within 3 megacycles from its centre frequency. In the latest design the conical pieces were made by the electrolytic processes. Thus it was possible to make them reasonably thin thus making the cavity tunable within certain limits.[8] The coupling between the resonator and the waveguide was found to be extremely critical to changes in the diameter of the coupling hole. The correct diameter was found empirically by progressively increasing the hole diameter until the desired degree of coupling was obtained. The machined hollow in the body of the cavity, between the cavity and the waveguide, illustrated at 'D' in the Fig. 4 was provided so as to avoid the necessity of feeding the resonator through a long coupling hole operating essentially in a cut-off mode. The absence of this groove would have necessitated the use of a coupling hole with a much larger diameter, resulting in a correspondingly larger disturbance of the field in the resonator. The various views and dimensions of the cavity are given in Figs. 3, 5, and 6.

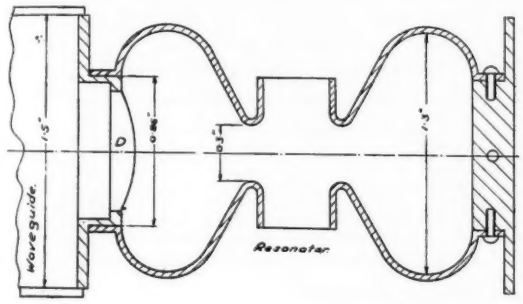

Fig. 4—Coupling of resonator to waveguide

5. Experimental Measurements

Frequency measurements were made by energizing the cavity from a highly stabilized tunable 10 cm. band Klystron (CV35), and measuring the relative response of the cavity over a range of frequencies including the resonant frequency, using extremely loose coupling.

The Q of the resonator and its circle diagram were obtained by measuring the impedance looking into the cavity over a similar range of frequencies with the standing wave method.[9] Rough and

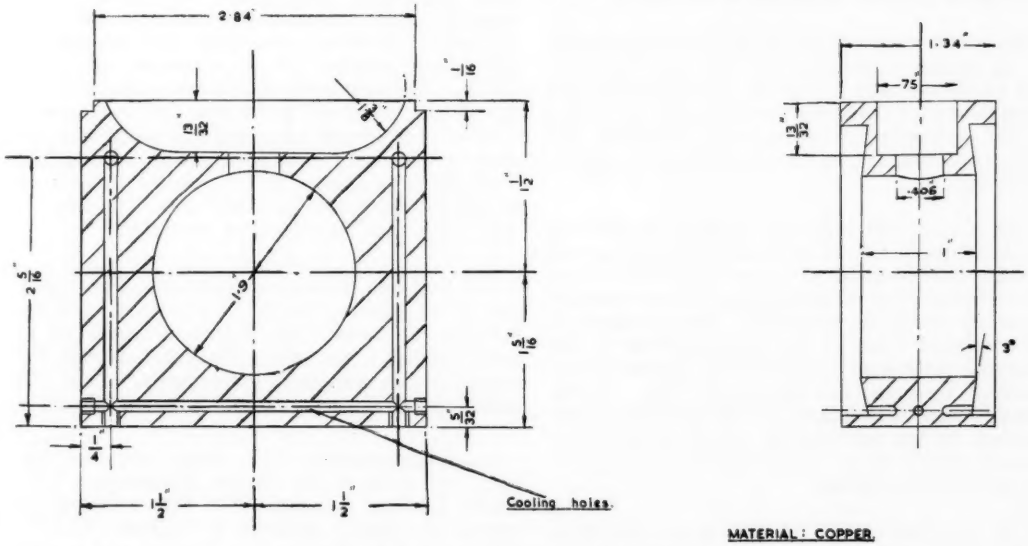

Fig. 5.—Cavity of resonator for 30 Mev. microtron.

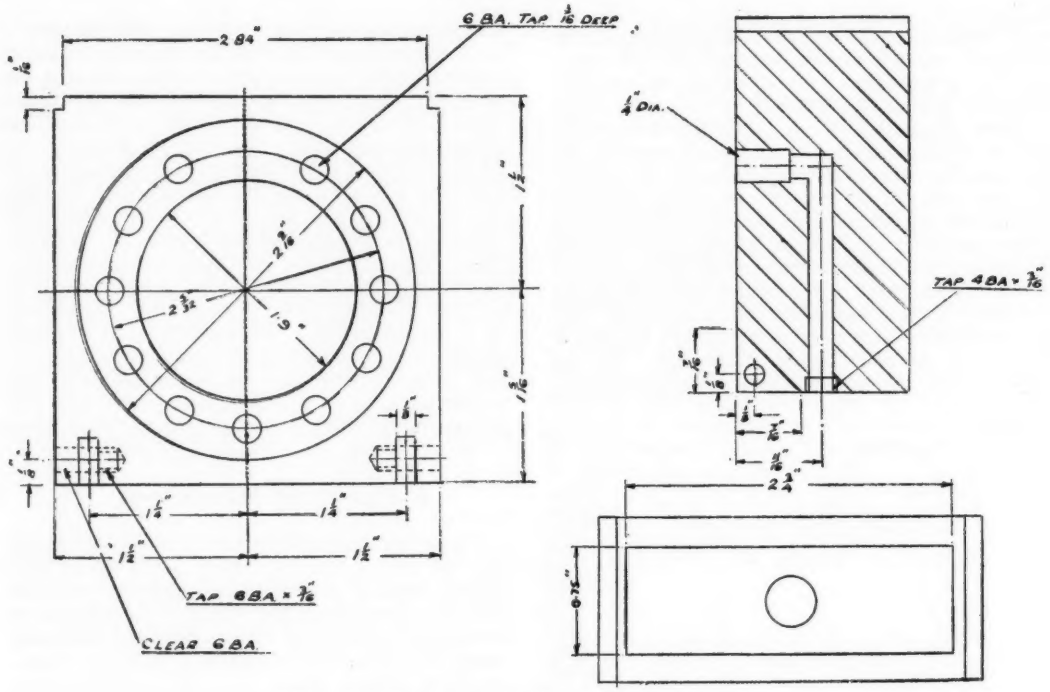

Fig. 6.—Cavity resonator for 30 Mev. Microtron.

quick estimation of the Q during experimental development of the cavity was made by a microwave cavity Q-meter described by LeCaine.[10]

The shunt resistance of the cavity was measured by the methods described by Hall and Parzen[11] and by Sproul and Linder.[12] Field distribution along the axis of the cavity was measured by moving a small dielectric ball along it and measuring the change in resonant frequency for different positions of the ball.

The plot of the difference between theoretical and measured values of resonant frequency against coupling hole diameter gave a smooth curve. From this curve the effect of the coupling hole on resonant frequency could be estimated. The addition of the estimated effect of the coupling hole to the measured value gave approximately the theoretical value of the resonant frequency. The results obtained were encouraging and the solution of the problem by the perturbation theory appeared to give reasonably accurate results so far as design of the cavity was concerned.

The measured value of the shunt resistance (0.71 megohms) for the silvered cavity however was approximately 50% lower than the theoretical value. The values of both Q and shunt resistance of the actual cavity constructed were lower than their theoretical magnitudes on account of a capacity formed between the cones across the gap. The results, however, were better than the theoretical values obtained for a re-entrant cylindrical cavity. The improvement of this cavity over the cylindrical re-entrant cavity was due to the introduction of re-entrant cones and also to the spherical shape of the discs to which the cones were attached. With this arrangement, it was possible to maintain a field pattern inside the cavity almost similar to that in a conical-line spherical resonator.

The field distribution along the axis of the cavity across the gap was found to be perfectly uniform between the cones. The field was attenuated to zero within 0.3 cm., along the holes through the cones, as we expected theoretically. Thus the radiation loss through the holes is practically nil.

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UNSAPONIFIABLES IN FATS: QUANTITATIVE DETERMINATION

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Unsponfiables in fats have been investigated and a simple apparatus for countercurrent extraction has been developed. Quantitative determination of unsponifiables together with that of the total content of fatty acids and esters have been made.

The unsaponifiables, non-fatty impurities, are always present in certain percentages in fats. These create problems both in fat and food chemistry. The various types of substances in the unsaponifiables, such as sterols, phenols, hydrocarbons, fatty alcohols, waxes, vitamins, antioxidants, coloring matters and indicidental impurities, if not properly removed or treated to eliminate harmful effects, may cause complication in the human system through continuous ingestion along with foods. The fats and fatty impurities are also known to be at the root of faulty fat metabolism, [1-3] leading to serious consequences of atherosclerosis and perhaps heart failure. [4-5] The best procedure adopted in this investigation for tackling the problems of unsaponifiables was to determine their exact quantities. A very simple apparatus for countercurrent extraction has been developed and used in place of the official manual procedures of A.O.C.S.[6] and Agricultural Chemists[7] (A.O.A.C.). As usual with the unsaponifiables, the contents of total fatty acids (TFA) and total fatty ester (TFE) have also been determined.

The prerequisites taken as guide in developing the apparatus were as follows: (*a*) the extractor is simple to construct and easy to maintain; (*b*) it is low in cost; (*c*) the extractor may lend itself to a wide variety of applications; and it eliminates emulsion problems often encountered in manual procedures.

Extractor

The apparatus illustrated in Fig. 1 is self-explanatory. Despite its apparent simplicity, there may be difficulties in the sequence of setting up the apparatus. It is to be noted that the procedures described in the text are to be followed in the right order. The optimum conditions for the most efficient extraction require a balance between minimum agitation and maximum solvent dispersion. Vigorous agitation may give emulsions and certain amounts of carry-over of undesirable portions. The inner-tube tip must be over the magnetic bar for efficient extraction. A magnetic bar (1" in length) will disperse the solvent without setting up a vigorous agitation at the solvent interface. The height of the tube above the 1-litre (wide-mouth) bottle and below the outlet arm, is critical in the sense that the column of solvent in this portion helps better separation of emulsion. The onset of emulsification can be controlled through experience by addition of small amounts of alcohol (2-3 ml. at a time) over a short period of time. Each fat may need special treatment according to its composition. Rubber stoppers are used for convenience. They are heated with 20% alkali for four hours and washed free from alkali before use. Our experience indicates that the rubber stoppers should be replaced every year. Ground-joints may be used instead, if possible.

Quantitative Determination of the Unsaponifiables

The procedures through the extractor developed are applicable to any type of fat. For a fat with high

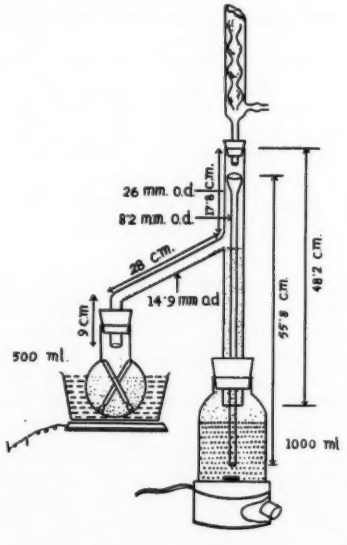

Fig. 1.—Extractor for determination of unsaponifiables.

amounts of unsaponifiables (over 7.0%) a slight increase in the alcohol percentage of the extractable solution, as noted in the foregoing section, may solve the emulsion problems. Three types of solvents, petroleum ether, ethyl ether and mixed solvent of petroleum ether+ethyl ether (1: 1) are chosen for trial in extraction. The mixed solvent being most efficient was employed in the procedures described below. The alcohol contents in soap stock solution for having no emulsion with the solvents are: petroleum ether, 33.0-37.5%, ethyl ether, 14.0-20.0%, and mixed solvent, 24.0-26.0%.

Apparatus and Reagents

The main apparatus as shown in Fig. 1; Erlenmeyer flasks, 250 ml., 2 separatory funnels, each 1 liter in capacity; 1-liter suction flask; and 50 ml. Erlenmeyer flask. Ethyl alcohol (absolute) distilled over KOH pellets and powdered zinc; aqueous potassium hydroxide solution, 100% KOH by weight (117.5 g. 85% KOH pellets in 100 ml. H_2O); ethyl ether (absolute) distilled over KOH pellets; petroleum ether (35-75°C.) distilled over KOH pellets; sodium hydroxide solution, 0.02N accurately standardized; and phenolphthalein indicator solution, 1.0% in 95% alcohol.

Procedures

An amount of fat between 9.0-10.0 g. was weighed accurately into the 250 ml. Erlenmeyer flask. To this was added by means of a syringe 80 ml. absolute alcohol, and 5 ml. of aqueous KOH. This was boiled steadily under a reflux condenser for one hour. Complete saponification was thus ensured.

The mixed solvent, ethyl ether+petroleum ether (1:1, 1000 ml.) was agitated with 24% alcohol (1250 ml.) and two phases were saved ($ether_2$ and $alcohol_2$ as stock solvents). The magnetic bar and 50 ml. $ether_2$ were introduced into the extraction bottle (Fig. 1) which was clamped to the rack of ring stands used for the extraction apparatus. The soap was carefully washed into the bottle with required portion of $alcohol_2$ (soap layer occupying 3/4th of the volume in the bottle) so that two layers resulted on stirring by magnetic system. $Ether_2$ was then added to bring the level to the neck of the bottle, without touching the stopper. The glass bumper and 500 ml. $ether_2$ were put into the stoppered 1/2-liter flask (Fig. 1) while setting up the extraction apparatus (without the condenser). Enough $ether_2$ was added to bring the level of solvent over the side arm. Now the condenser (with cold water flowing) was inserted in place, followed by necessary heating and stirring. Time for extraction with a heavy reflux (short of flooding) was limited to five hours.

The extract solution was carefully transferred to a 1-liter separatory funnel and washed three times with 100 ml. portions of 10% alcohol in distilled water. The first one was only swirled and the latter two were shaken. The combined wash liquid was extracted in the second separatory funnel, two times with 100 ml. portions of $ether_2$. This second extract was washed twice with distilled water.

The combined extracts were again twice washed with distilled water and dried over anhydrous sodium sulfate. The solvent was evaporated in 1-liter filtering flask under suction with a glass bumper (Fig.1) to a few ml. remaining. This was carefully transferred with ethyl ether to a 50-ml. Erlenmeyer flask and evaporated to dryness on steam of a water-bath under a gentle stream of clear and dry air. The drying was completed to constant weight. The flask with the unsaponifiables was warmed to 110°C. for five minutes in an oven and put while hot into a vacuum desiccator that was later subjected to the suction of a water pump for two hours and weighed. The vacuum oven drying method may also be used, if available.

The analysis and calculation were completed according to A.O.C.S. official method[8] which runs as follows :

After weighing, the residue was taken up in 50 ml. of warmed (ca. 50°C.) 95% alcohol containing indicator and previously neutralised to a faint pink colour. It was then titrated with 0.02N NaOH to the same colour.

$$\text{Weight of fatty acids in the extract, in g.} = \text{ml. of 0.02N NaOH} \times 0.0056$$

$$\text{Unsaponificable matter, \%} = \frac{(\text{Weight of residue} - \text{weight of fatty acids})\ 100}{\text{Weight of sample}}$$

Results and Discussion

Quantitative estimation of the unsaponifiables is very important, before their nature is studied. The procedure developed in the present investigation is helpful for determination of the unsaponifiable contents in several fats of economic value in East Pakistan. Table 1 shows the comparative results from the extractions by three solvents. The mixed solvent consistently gave better results. Consequently, this solvent was used in the present investigations for the extrac-

TABLE 1.—EFFICIENCY OF SOLVENT IN EXTRACTION OF UNSAPONIFIABLES.

Samples		Petroleum ether (% by wt.)	Ethyl ether (% by wt.)	Petroleum ether + ethyl ether =1:1 (% by wt.)
Hydrogenated cottonseed oil	*a.*	0.53	0.90	1.23
	b.	0.57	0.94	1.19
	c.	0.55	0.97	1.17
Sesame oil		1.83	3.18	3.56

TABLE 2.—UNSAPONIFIABLES IN DIFFERENT FAT SAMPLES.

Fat samples	Unsaponifiables	
	Percentage in fat	Presence of steroids
1. Market puti fish oil, (plain heating)	1.58	++
2. Solvent-extracted puti fish oil	2.35	++++
3. Oil from hilsa fish heads (solvent)	1.25	++++
4. Safflower oil ..	1.7	+++
5. Coconut oil ..	1.25	++
6. Coconut cake oil ..	2.20	+++
7. Sesame oil ..	3.56	++
8. Sesame cake oil ..	4.23	+++
9. Tamarind seed oil ..	3.80	++
10. Titgila oil ..	4.08	++
11. Groundnut oil ..	1.45	++
12. Mustard cake oil ..	4.85	+++

tion procedures. Table 2 summarizes the data on unsaponifiable contents which gave tests for steroids.

The main feature of the extractor is that it can be set up under any conditions of the laboratory; one-litre wide-mouth reagent or other bottle, rubber stoppers, glass tubes, half-a-litre flask, one small plate heater and one condenser are easily available. The magnetic stirrer may be arranged with one-inch magnetic bar and another rotating magnet (horse-shoe or otherwise) over a controlled motor underneath the bottle.

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CEREALS AND CEREAL PRODUCTS: PROPERTIES OF CERTAIN STARCH VARIETIES AND THEIR SOURCES IN EAST PAKISTAN

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The properties and other data of fourteen sources of starch have been described. The starch content and the particle size for each type of the isolated starch have been found to be quite satisfactory for commercial exploitation.

Introduction

Cereals being our staple food, starch for industrial uses, such as sizing materials, production of adhesives, syrups and glucose, must be obtained from other sources of less importance. Such sources have to be so chosen as to contain sufficient quantity of starch which would have properties necessary for commercial purposes. The decision needed numerous analytical experiments on different starchy products. Fourteen varieties such as (a) Mango seed (*Magnifera indica*); (b) Jack fruit seed (*Artocuspus integra*); (c) Mukhi Kachu (*Colocasia antiquorum*); (d) Man Kachu (*Alcocasia indica*); (e_1, e_2) Pani Kachu (*Aqua Casia indica*), Narkali Kachu (*Cococasia indica*); (f) Ol Kachu (*Amorphophallus campanulata*); (g) Cassava (*Manihot utilissima*); (h) Shati (*Zedoary curcam*); (i) Bhet (*Nympheca stellata*); (j) Singhara (*Trapa bispinosa*); (k) Makhna (*Euryale ferox*); (l) Mete Alu (*Diosoorea bulbifera*); (m) Kaon rice (*Setaria itelica*) satisfied our requirements, and their properties and other data are recorded in this paper. The starch contents and particle size for each type of isolated starch have been found to be quite favourable for commercial exploitation. As routine, the bye-product of starch was also obtained from (n) defatted ground-nut (*Arachis hypogea*) meal for analysis.

Experimental

Different varieties of the same sample were collected from the local markets. Their ash, protein, fat, fibre, starch and reducing sugar contents were determined by the methods of A.O.A.C.[1] Analysis of sodium and potassium was made gravimetrically,[2] that of calcium, volumetrically,[3] and those of phosphorus and iron, colorimetrically.[4]

Pure starch was isolated from the materials by the following method. The substance was first washed with water to remove the adhering dirt and then rasped mechanically to a pulp, whereby the cells were broken and starch grains exposed. The mass was thoroughly washed with water and then subjected to seiving through the fine brass-wire sieves which retained the coarser particles of cellulose but allowed the smaller starch granules to pass through. The milky, aqueous suspension, containing practically all the starch and some cellulose, was allowed to stand; the starch settled at the bottom, and the lighter particles of cellulose, etc. were scraped off from the top. The mass was again treated with fresh water and allowed to settle. The supernatant liquid was then decanted off. The starch was centrifuged to remove water and finally dried under vacuum without any heating, and subjected to photomicrographic studies, standardized through several trials with known starch samples from potato, rice and wheat. During the microscopic examination the dimensions of starch particles were also determined.

From Table 1, it is clear that all these food materials except ground-nut are good sources of starch; because their starch content varies from 50 to 91% on dry weight basis. Their ash, protein, fat and fibre content ranges from 0.51 to 5.8%, 2.85 to 12.49%, 0.36 to 2.35% and 1.51 to 12.11%, respectively. These ranges compare favourably with those standard sources like potatoe, rice and wheat. Sodium of all these materials is negligible; and their potassium content is also low, i.e. it varies from 0.0 to 2.9%. They also contain considerable amount of calcium and phosphorus, though their iron content is low.

Table 2 represents some characteristic features of the starch samples and their sources.

The photomicrographic features noted below do suggest grain-sizes, quite suitable for various commercial exploitations.

Photomicrographic features are shown in Figs. 1(a-n).

Generally mango-seed starch (a) is found as a mixture of irregularly oval and round granules. The oval or egg-shaped are usually larger and measure 28 micron; and the smaller are round and measure about 8 micron, the shape of tapering rods. The hilum is distinct and concentric.

Granules of jack-fruit seed starch (b) vary from 18 to 5.5 micron, some are round, some are angular and polyhedral. Hilum is distinct and rings are not visible. Their maximum dimension is 18.7 micron and minimum is 5.5 micron. The granules of Mukhi Kachu starch (c) are very small in size and their range is from 5.5 to 1.4 micron. They are more angular than rice starch particles. The majority of the particles are polyhedral. Their central hilum is well-developed, fissure is star-shaped and rings are not visible.

The granules of Man Kachu (d) starch have a great resemblance with those of Mukhi Kachu

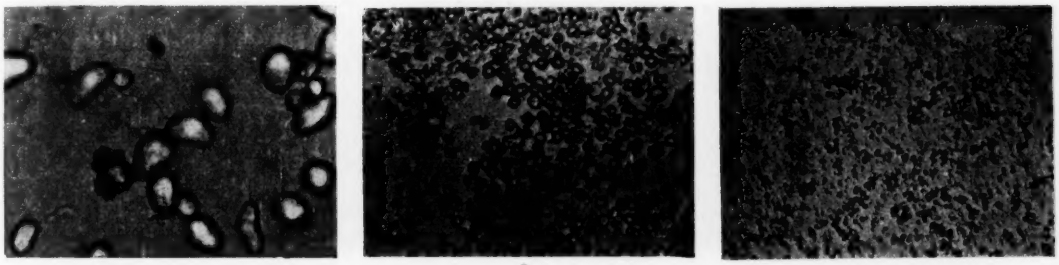
TABLE 1.—COMPOSITION ANALYSIS OF THE STARCHY PRODUCTS.[a]

Local Name	Water[b] g.	Ash. g.	Protein g.	Fat g.	Fibre g.	Reducing sugar g.	Starch g.	Minerals				
								Na_2O g.	K_2O g.	P g.	Fe g.	Ca g.
Mango seed	10.12	2.91	4.11	8.10	3.22	2.11	70.10	1.21	0.92	0.173	0.005	0.048
Jack fruit seed	62.42	4.13	12.41	1.26	3.12	2.25	75.61	Trace	2.05	0.181	0.016	0.082
Mukhi Kachu	71.24	3.31	8.75	0.36	11.01	1.51	70.23	,,	2.12	0.333	0.023	0.106
Man Kachu	83.12	5.80	10.7	2.35	9.50	1.60	68.51	,,	2.90	0.138	0.009	0.310
Pani Kachu	84.4	2.38	5.42	0.56	12.11	15.62	50.12	,,	1.03	0.125	0.007	0.251
Narkali Kachu	91.12	4.72	6.11	0.61	10.52	22.22	47.70	,,	2.40	0.285	0.009	0.259
Ol Kachu ..	71.24	3.31	8.75	0.36	11.01	1.51	70.23	,,	1.68	0.153	0.007	0.095
Cassava ..	—	2.63	2.85	0.75	2.81	1.12	91.30	,,	1.28	0.212	0.005	0.116
Shati ..	9.83	1.62	1.21	0.52	1.80	1.51	83.24	nil	nil	0.833	0.014	0.077
Bhet ..	80.17	0.72	10.8	1.88	9.25	2.17	71.14	Trace	nil	0.133	0.002	0.047
Singara ..	76.12	3.63	8.62	0.72	4.51	1.61	82.10	,,	1.58	0.444	0.412	0.211
Makhna ..	—	0.51	12.49	0.55	1.51	0.91	89.68	,,	0.11	0.006	0.003	0.059
Mete Alu ..	81.19	3.69	10.25	1.28	7.05	2.51	71.21	,,	2.01	0.210	0.004	0.214
Kaon rice	10.10	1.61	10.51	1.15	1.95	1.21	75.10	,,	0.25	0.040	0.021	0.109
Ground-nut (cake)	12.8	5.2	40.6	7.5	9.6	—	15.0	,,	Trace	—	—	—

a. Calculated on the basis of dry weight. b. Calculated on the basis of fresh weight.

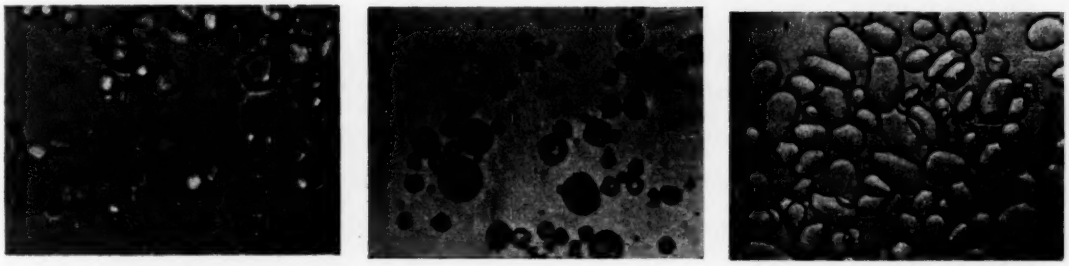
TABLE 2.—SOME CHARACTERISTIC FEATURES OF STARCH SAMPLES AND THEIR SOURCES.

Name of starch source	Feature	No. of sample analysed	Average wt. for each g.	Kernel %	Coat %	Dimension of the starch particles			
						Maximum size Micron	% of total number	Minimum size Micron	% of total number
Mango seed ..	Seed	7	22.5	72.2	27.8	28.1	75	8.3	25
Jack seed ..	Seed	8	7.1	97.5	2.5	24.4	50	7.0	50
Ol Kachu ..	Corn	5	2500	91.9	8.1	18.7	60	5.5	40
Mukhi Kachu ..	Corn	6	15.02	97.1	2.9	5.5	50	1.4	50
Man Kachu ..	Rhizome (root stock)	7	9500	85.2	14.8	4.6	50	1.3	50
Pani Kachu ..	,,	7	6512	86.2	13.8	—	—	—	50
Narkali Kachu ..	,,	7	6500	86.2	13.8	—	—	—	50
Mete Alu ..	Bulbil	5	950	92.1	7.9	58.2	20	12.5	80
Bhet ..	Seed	8	48.5	60.5	39.5	7.5	50	2.2	50
Singara ..	Fruit	7	10.5	81.9	18.1	39.0	70	15.1	30
Shati ..	Rhizome	8	12.2	97.1	2.9	62.9	25	22.3	75
Cassava ..	Root	8	512.6	94.1	5.9	18.7	50	22.3	50
Kaon rice ..	Seed	7	—	98.1	1.9	17.2	20	6.5	80
Makhna ..	Seed	8	120.1	80.0	20.0	3	50	1	50
Ground-nut ..	Nut	8	7.2	85.1	14.9	13	40	4.4	60

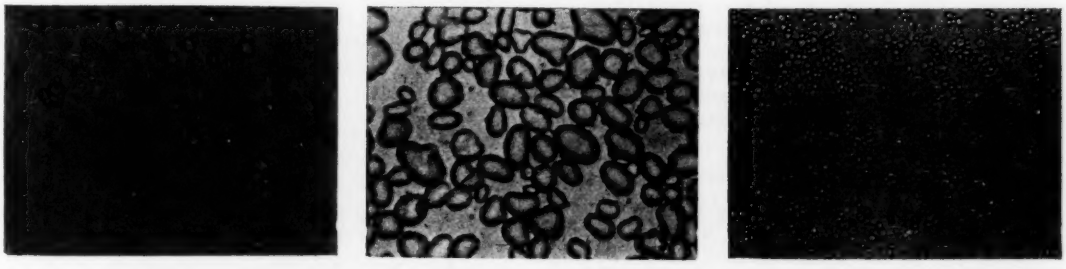


(a) Mango seed (b) Jack fruit seed (c) Mukhi Kachu

(d) Man Kachu (e_1) Pani Kachu (e_2) Narkali Kachu

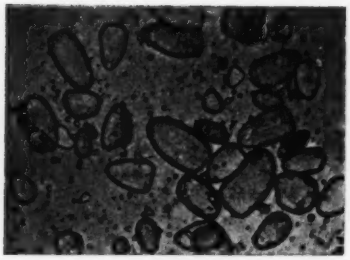


(f) Ol Kachu (g) Cassava (h) Shati

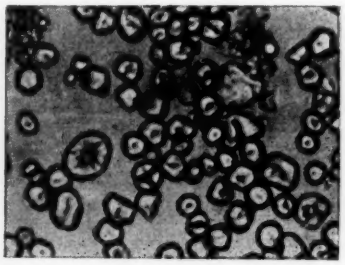


(i) Bhet (j) Singhara (k) Makhna

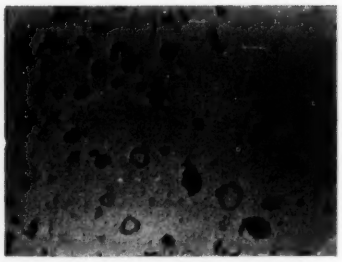
Fig. 1.—Photomicrographs of starch granules of different varieties (continued on next page).



(l) Mete Alu



(m) Kaon rice



(n) Ground-nut

not only in shape but in size also. As in Mukhi Kachu the dimension of the Man Kachu starch particle varies from 4.6 micron to 1.3 micron.

The shape of the Pani Kachu (e_1) and Narkali Kachu (e_2) starch particles are almost round and are often massed in small clustres. They are too small to be measured. Neither rings nor hilum are visible.

O1 Kachu starch (f) granules are varied in shape. Some of them are quite round, some have one end cut-off, leaving a concave or flat suface. But some of them also are polyhedral. The majority of the particles are small. Their dimension varies from 18.7 to 5.5 micron.

Cassava (g) granules are round or oval in shape with an indentation on one side which is characteristic of this starch. In polarized light a well-developed cross is observed. The size of the granules varies from 18-22 micron.

The majority of Shati starch (h) particles are pear or conical in shape with a few of round shape. But almost all of them have angular cones on both sides. The rings are concentric and hilum is not visible. The dimension of the maximum is 62.9 micron and that of minimum size is 22.3 micron.

Granules of Bhet starch (i) are similar to Mukhi Kachu starch. They are quite angular, the majority being polyhedral. There is a central hilum and rings are not visible. Their dimension varies from 7.5 to 2.2 micron.

Singhara starch (j) particles closely resemble potato starch in shape. Most of them are oval having one or two nodule on both sides. Some of them are almost spherical also. No hilum is apparent, rings are not visible. Dimension varies from 39.0 to 15.1 micron.

Makhna starch (k) granules are smallest of the starches found. Their size is from 1 - 3 micron. They are spherical with distinct central hilum.

Mete Alu starch (l) particles are regularly triangular in shape. The majority are of maximum size. No hilum or rings are visible. Their maximum dimension is 58.2 and minimum is 12.5 micron.

The shape of Kaon rice (m) starch is quite variable. Some of them are spherical in shape, some are angular and polyhedral. Their central hilum is distinct, fissure is star-shaped and rings are not visible. The dimension of the particles varies from 17.2 to 6.5 micron.

Ground-nut (n) starch granules are spherical in shape. Their hilum and rings are not visible. The upper limits of size are usually 13 to 10 micron and lower limits are about 4.4 - 5 micron.

Acknowledgement—The technical assistance of Mr. A. M. M. Ahad for mineral analysis and that of Mr. M.N. Haque and Mr. M. Rahman for taking the photomicrographs of the starch granules is acknowledged. Thanks are also due to Dr. M.M. Qurashi and Mr. A.K.M. Ahsanullah for help in the initial preliminary work at the beginning of 1958 season on photomicrography of certain starch granules.

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A STUDY OF WATERMELON RUST

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A new disease, the rust of watermelon, first observed in 1949, seems to be endemic to the region and as yet confined therein. Although the fungus *P. citrulli* is specifically identical with the one occurring elsewhere on coloycnthis (*C. colocynthis*), it is considered to be a different variety and named *P. citrulli* variety *valgari*. The pathogen has been seen to enter both through the stomata and through the cuticle. The teleutospore have been found to need a long resting period and to germinate only if subjected to freezing and thawing for a period of 45-60 days. So far the method of perennation remains unsolved. The possibilitiy of an alternate host being responsible is being investigated.

Introduction

The rust disease of wetermelons was first observed at Karachi in the course of a routine survey of plant diseases in the outlying region of Malir.[1] The disease was severe enough in that year as well as in 1950, to materially decrease the yield as well as the quality of watermelons in the affected areas. In fact, some of the fields were so heavily affected in 1950 that the owners were advised to burn up the affected crop. At the same time, a vigorous compaign of dusting and spraying was undertaken and the disease brought under control. However, there have been occurring since then outbreaks of this rust in these same areas of Malir and Landhi, off and on, in various years.

Curiously enough, even though the crop of watermelon is sown all over the Sind and Punjab to the north, and in the Rajisthan in India to the east, there have been no report of the occurrence of watermelon rust in any of these areas. Indeed as far as is known the disease is endemic to Karachi, even though the allied plant *Citrullus colocynthis* was reported some fifty years ago as being parasitised by a rust named *Puccinia citrulli* by Butler[2] as far down as Madras in the Indian peninsula.

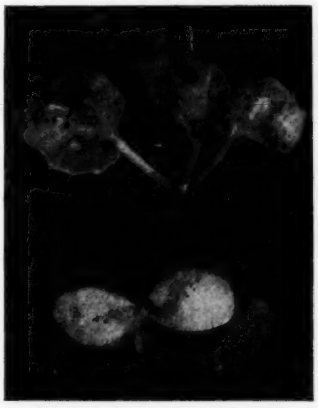

Plate 1.— Formation of uredial sori on the leaves of colocynthis (*Citrullus colocynthis*).

In view of the importance of the crop, an intensive investigation of the disease was undertaken in 1957-58 to determine the variety of the parasite, its mode of infection, perennation and multiplication, collateral host, if any, and to isolate any variety of watermelon that may be resistant.

Uredospores of the fungus were taken from the natural local host, *Citrullus vulgaris* Linn. and inoculated on *C. colocynthis Schrad.* using the technique detailed elsewhere.

The inoculated plants of *C. colocynthis* after the usual period of incubation produced sori (Plates 1 and 2) which were similar to those on *C. vulgaris.*

These experiments were repeated four times successfully. The results, summarised in Table 1 below, definitely indicate that the rust observed to occur at Karachi on *C. vulgaris* is specifically the same as the one reported by Butler from Madras occurring on *C. colocynthis,* namely *Puccinia citrulli.*

However in spite of careful search, no colocynthis plants have been found infected in nature with this rust, even on plants growing in the fields of watermelon heavily rusted. It seems reasonable to conclude that while in artificial inoculations, rust on watermelon can be successfully transferred on to the colocynthis plant, the same is not the case in nature.

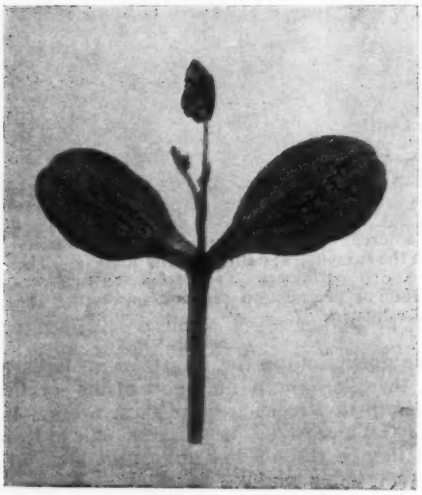

Plate 2.—Formation of uredial sori on the leaves of watermelon (*Citrullus vulgaris*).

TABLE 1.—SIZE OF UREDOSPORE IN MICRONS, OF *P. Citrulli* ON THE HOST.

On *C. vulgaris* collected from Malir	On *C. vulgaris* inoculated in Labs.	On *colocynthis* inoculated in. Labs	On *C. colocynthis* as reported by Butler
Length :			
23.10 - 29.70	23.10 - 29.70	22.38 - 29.52	20 - 30
Breadth :			
19.80-24.75	19.80 - 24.75	19.98 - 24.08	18 - 24

This conclusion is strengthened by the fact that areas where the rust has been reported on colocynthis[2] have not reported it on watermelons.

In spite of specific identity therefore it is reasonable to regard the rust on watermelons as belonging to a variety distinct from that occurring on colocynthis. For this former, which is the subject of present investigation, the name *Puccinia citrulli* var. *vulgari* (var. novo) is proposed to distinguish it from *Puccinia citrulli var. colocynthi*.

Perennation of the Fungus

The following could be the possible sources of perennation through the uredial stages:

1. Volunteer plants of watermelon.
2. Collateral hosts.
3. Old plant debris.
4. Migration of spores from the adjoining areas.
5. Some alternate host producing aecia and aecial spores.

1. *Volunteer Plants.*—The possible perennation of the fungus in the field through volunteer plants has been studied during the years 1956-57, 1957-58 and 1958-59. Off-season plants of watermelon were kept under weekly observation in the field previously infected by the fungus but no case of infection could be detected on these plants till the main crop had come into its own from April to June. Only rarely the infection was carried up to the first week of July, even though there is an abundance of watermelon plants both before and after this period. Volunteer plants therefore do not seem to play any significant part in the perennation as far as our present observation goes.

2. *Collateral Host.—Citrullus colocynthis* which is seen growing through out the year has also been kept under constant observation month by month but as described elsewhere it has never been found attacked by this fungus in the field, even when growing in close proximity of watermelon plants and in those same fields where there is year after year fungus infection in the month of April, May, and June. It is only under controlled conditions that *C. colocynthis* has been found to act as a collateral host as described above. Colocynthis therefore does not seems to act as collateral host for this rust in nature.

3. *Old Plants Debris.*—In late June and July after the majority of the fruits have matured and before the crop dries out completely, some of it is fed to the cattle, and the rest usually just left over in field. During this season the temperature often shoots up from 112°F. to 118°F. in the shade and the plants soon dry up. The dried up debris is then ploughed in the field for manure. Thus there is not much plant material left over, which could act as source of infection. However some of the rusted plant debris was kept under room temperature in the Laboratories, and the spores were taken therefrom week after week and studied for their viability. It was noted that material which was collected on 2nd June had the viability of 75% of spores when tested the same day, but after two days only 10% spores were viable. After ten days when the material was tested on 12th June, 1959 there were no viable spores. It thus seems that plant debris either could not serve as source of uredo spore infection from one season to the other.

4. *Migration of Spores from Adjoining Areas.*—In areas adjoining Karachi, i.e. Baluchistan and Sind, watermelon crop has two seasons of cultivation: (a) March to June and (b) August to

October. In none of these areas however the disease has ever been reported either on the watermelon or on colocynthis. In fact from all evidence so far available the fungus seems to be endemic to Karachi on the watermelon only; thus there appears no outstanding source for the spores or spore material to be brought to Karachi to start the infection. Further, the prevailing direction of wind during this season is south westerly, i.e. from the sea, thus the wind blows from Karachi to Sind rather than in the opposite direction.

The perennation of the fungus in Karachi area is thus still something of a puzzle. Further studies including those on the role of possible alternate hosts might solve the riddle.

Seasonal Variation in Infection in Field

The watermelon crop at Karachi is normally in the field from March to July. A survey of the crop was therefore undertaken during the season and the extent of infection recorded month by month. A summary of these observations is given in Table 2.

TABLE 2.—SEASONAL VARIATIONS IN WATERMELON RUST INFECTION IN THE FIELD.

Aug. to Mar.	April				May				June				July		
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3
	←						Weeks								→
No crop															
Traces or nil	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Very slight	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Slight	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Mild	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Mildly epiphytotic, all fields					.	.	.	.	.	.	.	.	.	.	.
Moderately epiphytotic									.	.	.	.	.	.	.
Severe									.	.	.	.	.	.	.
Severe									.	.	.	.	.	.	.
Very severe									.	.	.	.	.	.	.
Very severe									.	.	.	.	.	.	.
Mild; infection dying out									.	.	.	.	.	.	.
Slight													.	.	.
Very rare													.	.	.
Nil													.	.	.
Nil													.	.	.

The fall of infection at the end of June was due to the uprooting of plants as the fruits matured by the 3rd week; in 4th week the rains started. After the rains the infection comes to an end.

Varietal Susceptibility

Several varieties of watermelons are cultivated commercially. These are chiefly distinguished by the colour of the ripe fruit and of seed. Some of these varieties have been tested for possible variations in susceptibility to the rust. All these commercial varieties were found as highly susceptible as one cultivated locally. Results of these experiments are given in Table 3.

Signs and Symptoms

The uredia and the telia are produced mainly on leaves, but occasionally also on the petiole and stem. The uredia while occurring on both sides of the leaves are commonest on the upper surface. As the mycelium extends through the thickness of the leaf, it is common to find a pustule on the upper surface and vice versa. When uredia are few and not crowded it is customary to find a ring of smaller uredia around a larger central one (Plate 5).

Uredia are without spot, scattered, round, 0.5 to 2.5 mm. in diameter. With the increasing intensity of infection, the pustules formed are generally smaller in size than at the beginning of the infection. At first they are sub-epidermal but soon the epidermis ruptures exposing the powdery masses of spores. When uredia first appear, there is usually little or no discolouration of the surrounding leaf tissue, but as the infection proceeds and especially when sori are numerous the leaf becomes partially or completely yellowed and dies. In some cases it has been noticed that the sori are surrounded by partially discoloured areas of leaf, in some cases typical necrosis takes place (Plate 3). Whether these reactions could be indicative of the presence of more than one physiological race in the population has not yet been ascertained.

TABLE 3.—SUMMARY OF THE RESULT OF INOCULATION ON DIFFERENT VARIETIES OF WATERMELON.

	Karachi black	Karachi green	*Citrullus colocynthis*	Sind black	Sind green with red seed	Sind green	Punjab black	Punjab green
No. of plants inoculated	450	100	100	50	30	30	350	100
No. of plants got infection	450	100	100	50	30	30	350	100

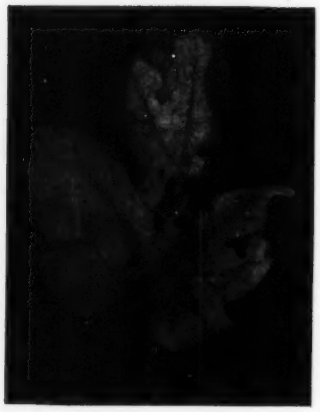

Plate. 3.—Necrotic zones on the leaf of watermelon (*Citrullus vulgaris*).

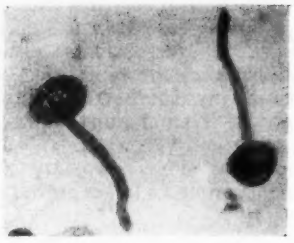

Plate. 4.—Germinating uredo spores showing hyaline germ tube.

Uredia at first are yellowish brown, later they change to brown or slightly dark brown. Telia are dark brown in colour, occur both on dorsal and ventral surfaces of the leaves. They are minute, rounded, usually mixed with uredia and very occasionally separate. The formation of telia does not seem to be general. It seems to depend upon a combination of various climatic conditions, e.g., higher temperature accompanied by low humidity.

Nature of Pustules

The pustules are identical on both the surfaces. Small uredia may be surrounded by necrotic zones. At first the leaves show only a little necrosis but later it becomes more clear, and the leaves become more chlortic and die prematurely. In some cases two or three sori which are broadly scattered show a whitish or discoloured area surrounding them together. After a few days a ring of numerous small pustules is produced around it (Plate 5). Occasionally the tissues where the sori are formed is raised due to the excessive growth in the leaf tissue resulting in the small cavity below the raised portion. The arrangement of sori is irregular, i.e. scattered on the whole surface.

Plate 5.—Ring of small uredia around the larger central one.

The uredo spores are 23.10-29.70X19.80-24.75 M (cf. Table 4). They are yellowish brown to light brown in colour, spherical or ovate in shape. There is a single thick echinulated wall with 4 equatorial germ pores. The spores germinate readily in water. At 14°C. it takes only two hours for germination of fresh spores giving a glassy hyaline germ tube (Plate 4). The germ tube is 6.6 M in diameter.

TABLE 4.—SIZE OF UREDOSPORES IN MICRONS.

Leaf collected from Malir			Leaf inoculated in Lab		
Length/ no. of spores	Breadth/ no. of spores	Total no. of spores	Length/ no. of spores	Breadth/ no. of spores	Total no. of spores
29.7/19	23.97/14	100	29.70/13	24.75/3	100
28.00/13	23.10/36		28.00/7	23.93/5	
27.20/6	22.28/7		26.40/40	23.10/40	
26.40/24	21.45/26		25.57/3	22.28/22	
24.75/19	20.60/10		24.75/16	21.28/22	
23.10/19	19.80/7		23.10/211	9.80/8	

Size, Colour and Form of Teleuto-spore

The teleuto-spores are 26.40-33.00X31.35-42.2 M (cf. Table 5). They are two-celled, dark brown to chestnut brown in colour, ellipsoid, slightly constricted in the middle, with a smooth thick wall. The germ pore of the upper cell is just at the centre ofthe apex, while germ pore of the lower cell is just

below the septum. Stalk is not very persistent and is 19.30 - 36.30X4.95 - 6.6 M (Table 6).

TABLE 5.—SIZE OF TELEUTO-SPORE (IN MICRONS).

Collected from Malir			Produced in Lab.		
Length/ no. of spores	Breadth/ no. of spores	Total no. of spores	Length/ no. of spores	Breadth/ no. of spores	Total no. of spores
42.20/9	33.00/8	100	42.25/12	29.70/67	100
3.60/22	31.35/6		39.60/49	28.00/17	
37.90/15	29.70/43		37.90/12	26.49/13	
36.00/27	28.00/12		36.30/24	26.00/3	
34.60/10	26.40/31		34.60/3		
33.00/13					
31.35/4					

TABLE 6.—SIZE OF TELEUTO-SPORE STALK (IN MICRONS).

Length/no. of spores	Breadth/no. of spores	Total no. of spores
36.30/8	6.6/81	100
33.00/5	5.77/9	
31.55/3	4.95/10	
29.70/21		
28.00/4		
26.40/16		
23.10/21		
21.45/20		
19.30/3		

Teleutospores have a long resting period and could only germinate if subjected to freezing temperatures. The present experiment on germination (Table 7) shows that the spores needed alternate freezing and thawing for a period of 45-60 days before they germinate to produce the promycelia.

Method of Inoculation

The viable uredo spores have been used successfully for inoculating plants (before inoculation the spores were tested for their viability). Inoculations were made by two different techniques :

(*a*) By the simple spraying of a spore suspension on leaves, and
(*b*) By injecting spore suspension below the epidermis with a hypodermic syringe.

TABLE 7.—GERMINATION OF TELEUTO-SPORE (SPORES KEPT IN REFRIGERATOR ON 9-7-1956).

Date of examination	Observation	% of germination
9-7-1956	No germination	0
15-7-1956	,,	0
21-7-1956	,,	0
2-8-1956	,,	0
9-8-1956	,,	0
16-8-1956	,,	0
24-8-1956	,,	0
30-8-1956	,,	0
9-9-1956	2 spores germinated	4

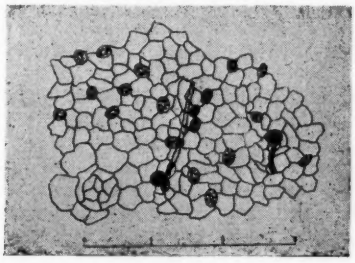

Plate. 6.—Path of infection through the cuticle.

After inoculation the plants were covered over by a bell jar, humidified and the plants together with the bell jar kept in a refrigerator for 16-24 hours at 14°C. Thereafter the bell jars were removed and the plants kept at room temperature and humidity. During winter with room temperature ranging from 24-16°C. or lower, it was not necessary to keep the inoculated plants in a refrigerator. Still the plants needed keeping under the bell jar for the sake of humidity.

The incubation period, i.e., the time from inoculation to the appearance of faint brown spots, was usually six days under the conditions of these experiments when temperature ranged between 26-32°C. and humidity was 100%. A further period of two days was required for the appearance of pustules. During winter when temperature falls down, the incubation period prolongs by one to two days depending on the fall of temperature.

Path of Infection

The uredo spore on germination on leaf surface gives rise to a germ tube which is unbranched

till it enters the host tissue. The germ tube usually enters the leaf through cuticle (Plate 6) but it also enters through stomata. In some cases, germ tubes were observed entering into the leaf through the basal cell of the leaf hair, and reaching through them into the pallisade tissue of the leaf.

Host-Parasite Relationship

After entering into the host tissue, the mycelium ramifies in all directions between and within the cells. At the time of spore formation, the hyphae collect below the epidermis and produce spores on small stalks which exert a pressure on the epidermis, resulting in its ultimate rupture and liberation of the spores.

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A STUDY OF THE ATOMOSPHERIC FUNGAL FLORA OF KARACHI CANTT.

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(Received May 20, 1960)

A survey of the fungal population of the atmosphere at Karachi has been made. During one year 44 species of fungi have been recorded. These include 6 species new to Pakistan and two quite new species of *Curvularia*. Five strains having ochraceous shades but belonging to *Aspergillis sulphureous* were also discovered.

In order to explain the appearance of spontaneous pathogenic as well as saprophytic infections of which the etiological factors are not known, the study of the fungal spores of atmosphere is of immense importance. Considerable attention has been paid to it by previous workers and the study of atmospheric fungal spores, in connection with the wheat rust, found great importance in India. It is considered of interest to study the nature and type of atmospheric fungi in relation to those which are isolated from the deteriorated material and it was found that, to a large extent, the deterioration was due to the atmospheric micro-organisms specially fungi.

The results of study of the atmospheric fungi over a period of one year at these laboratories in Karachi Cantt. from October, 1958 to September, 1959 are as follows.

Petri plate method was adopted for counting the atmospheric fungal population. Petri plates of 3½ inches diameter were used; 20 ml. of the Czapek's agar was poured in the sterilized petri plates under aseptic conditions and these were kept for about 4 days to test the sterility of the medium. The petri plates were simultaneously exposed for about 10 minutes against the wind current at 11 a.m. fortnightly at a height of 10 feet from the ground. The petri plates were incubated at room tempratures for seven days, and afterwards pure sub-cultures were made and specimens examined for identification. [2-5]

A summary of the results of observations on the total number of colonies in different months and the number of colonies in descending order are represented by Tables 1 and 2, respectively.

This clearly shows that the fungal population in May is the highest and in February the lowest. Fungal population in April and September, June and December, January, July and November do not differ considerably in themselves; February, and October give low figures than those observed in other months. It may be noted that there is a periodic rise and fall in the number of organisms.

Table 1 may be explained by a diagrametic representation of the fungal distribution in Karachi-Cantt. all the year round (Fig. 1). In this diagram the year is represented by a circle and the months are plotted at equal distance from one another. The fungal population is shown inside the circle by arrows from the respective months. The shaded portion from the middle of October to middle of February indicates winter. Summer is represented by the remaining circle out of which

TABLE 1.—FUNGAL POPULATION DURING DIFFERENT MONTHS.

1958			1959									
Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	G. total
10	14	20	15	6	22	25	28	20	14	17	24	215

TABLE 2.—FUNGAL POPULATION DURING DIFFERENT MONTHS IN DESCENDING ORDER.

May	Apr.	Sept.	Mar.	June	Dec.	Aug.	Jan.	July	Nov.	Oct.	Feb.	Total
28	25	24	22	20	20	17	15	14	14	10	6	215

the dotted portion (middle of June to middle of August) distinguishes the annual precipitation.

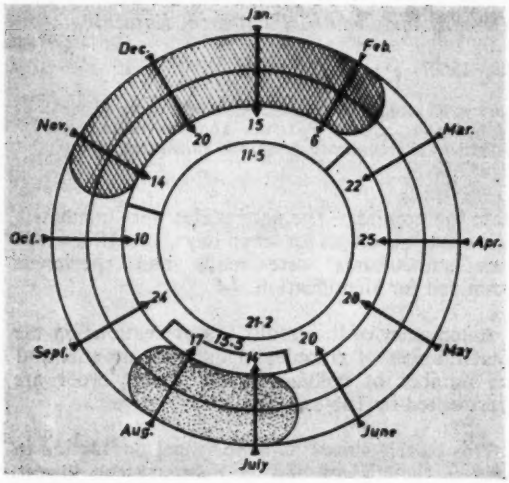

Fig. 1.—A diagramatic representation of the fungal distribution in Karachi Cantt. through the year.

The average of the colonies found in the winter is 11.5, in the summer including the rains it is 21.2, and in the rainy season it is 15.5. There may be two reasons which may explain the cause of the higher frequency of the fungal colonies in summer:

1. The wind velocity during summer is higher and so a greater number of the dust particles (to which the spores adhere) as well as the spores themselves strike the petri plates and therefore comparatively large number of spores are deposited on the medium surface.

2. During summer as the spore production in nature rises, the number of spores deposited on the medium surface naturally increases.

The winter gives comparatively poorer frequency of the fungal colonies, particularly because of the natural low spore production in cooler temperature in contrast to that in higher.

In the case of rainy season the fungal population lies between that of summer and winter which may

TABLE 3.—COLONIES OF DIFFERENT TYPES OF FUNGI.

S. No.	Organisms	Number of colonies	S. No.	Organisms	Number of colonies
1.	*Absidia sp.* ..	2	23.	*Fusarium solani.*	2
2.	*Actinomyces sp.* ..	5	24.	*Fusrium semitectum*	2
3.	*Alternaria sp.* ..	15	25.	*Fusarium dimerum*	2
4.	*Alternaria tenuis* ..	7	26.	*Fusarium equiseti*	3
5.	*Alternaria humicola* ..	2	27.	*Fusarium sporotrichioides*	3
6.	*Aspergillus candidus* ..	2	28.	*Helminthosporium sp.* (1)	6
7.	*Aspergillus flavus* ..	18	29.	*Helminthosporium sp* (2)	5
8.	*Aspergillus fumigatus.* ..	4	30.	*Helminthosporium sp* (3)	9
9.	*Aspergillus nidulans* ..	4	31.	*Humicola sp.* ..	4
10.	*Aspergillus niger* ..	17	32.	*Mycelia sterlia* ..	4
11.	*Aspergillus ochraceous* ..	7	33.	*Neurospora citophila*	5
12.	*Aspergillus stellatus* ..	2	34.	*Paecilomyces varioti.*	1
13.	*Aspergillus sulphureous* ..	2	35.	*Pencillium citrinum*	2
14.	*Aspergillus sydowi* ..	6	36.	*Pencillium sp.* (1)	5
15.	*Aspergillus tamarii* ..	3	37.	*Pencillium sp* (2)	2
16.	*Aspergillus terreus* ..	3	38.	*Pencillium sp.* (3)	1
17.	*Circinella sp.* ..	2	39.	*Phoma sp.*	2
18.	*Cladosporium sphaerosperumum*	10	40.	*Rhizoctonia sp.*	2
19.	*Cladosporium sp:* ..	13	41.	*Rhizopus arrhizus*	2
20.	*Curvularia sp. nov.* (1) ..	2	42.	*Rhizopus nigricans*	7
21.	*Curvularia sp. nov.* (2) ..	1	43.	*Trichoderma koningi*	2
22.	*Curvularia sp.* ..	8	44.	*Trichoderma Sp.*	3

be explained by the following factors :

1. Rainy season of Karachi comprises a part of the summer and accordingly production of spores in nature remains higher and so the number of fungal colonies is more when compared to that of winter.

2. During rains the dust particles settle along with the rain droplets decreasing the percentage of the atmospheric fungal spores and we get less fungal colonies compared to the dry months of summer but still higher than the winter.

Data on the total number of types of fungi identified are recorded in Table 3.

Forty four species of fungi were recorded during the entire period of exposure. Out of the fungi collected, *Aspergilli* are the most dominating. They are of common occurrence on the deteriorated materials like cotton textile, water-proof canvas and cordage etc. The other groups which were also found in abundance are *Alternaria, Helminthosporium*, *Curvularia* and *Fusarium* which are also responsible for the cellulose decomposition and are usually found on various cotton products deteriorated under exposed conditions. *Helminthosporium* is most abundant in the air and occasionally found on exposed cotton textiles.

Total number of new records of fungi during the atmospheric survey was 16. Out of these 9 species were such which were not reported from Pakistan previously and they are : *Absidia* sp., *Aspergillus stellatus*, *Circinella* sp., *Cladosporium sphaerospermum*, *Fusarium equiseti*, *Fusarium sporotrichioides*, *Humicola* sp., *Neurospora citophila* and *Paecilomyces varioti*.

Out of the remaining seven species two were of the genus *Curvularia* which were quite new species and have been dealt in separate papers. Remaining 5 species were interesting strains of *Asperigillus sulphureous* series. These strains were ochraceous in shade but several microscopic details pleaded their inclusion under *Aspergillus sulphureous* series. Their study was taken separately and an exhaustive paper is under publication.

Summary

A survey of the fungal population of the atmosphere was made for a period of one year and 44 species of fungi were recorded. Sixteen species new to Pakistan were reported. Out of these, two quite new species of *Curvularia* were encountered and five interesting strains, having ochraceous shades but belonging to *Aspergillus sulphureous* series were found. The fungal population showed a periodic rise and fall and also a significant numerical variation between months. Maximum number of fungal colonies were found during summer and minimum during winter which may be due to the difference in the wind velocity during these seasons and the difference in the production of spores in nature. Generally the same fungi are found in the atmosphere which have been encountered in the deteriorated cotton textile etc.

Acknowledgement.—The authors desire to record their thanks to Dr. Salimuzzaman Siddiqui for his help and suggestions. Thanks are also due to the Director, Commonwealth Mycological Institute, for his help in the identification of fungi.

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SOME INTERESTING STRAINS OF ASPERGILLUS OCHRACEOUS GROUP

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(Received May 20, 1960)

During a survey of the fungal population in the atmosphere at Karachi some interesting strains of *Aspergillus ochraceous* group have been discovered. It was found that the classification of ochraceous group, primarily on the basis of colour of the conidial heads is not always satisfactory. *Aspergilli*, having ochraceous shades when examined minutely, are found to be of sulphureous series rather than those of the ochraceous.

During the work on aerial fungi in these Laboratories five *Aspergillus* strains belonging to ochraceous group with ochraceous shades were collected. On microscopic examinations they resemble sulphureous series. The specimens were consequently sent to Commonwealth Mycological Institute, Kew, Surrey, England, for identification.

Detailed description of the strains follows.

A. Sulphureous: Strain No. 1

Fast growing strain (3.5 cm. in diameter at a temperature of 75°F.±2°F. by single spore inoculation), with abundant cream coloured conidial heads which change to ochraceous shade. No colouration on the reverse of the tube when young but brownish colouration appears in old cultures. At places cotton-fibre-like aerial mycelium present. Conidiophores vary greatly in length and diameter, rough and granulated, yellowish, specially in the outer layer (Fig. 1a). Conidiophores are wavy near both the ends. In old cultures the conidial heads break away leaving the funnel-shaped conidiophore ends (Fig. 1b). Metulae bear usually up to five phialides each (Figs. 1 d, e, f) but in the case of very small conidial heads which are occasionally seen, the number of phialides may be less (Fig. 1c). The conidial chains are very long in the old cultures and collectively form a broom-like structure. The conidia are globose, small and smooth; sometimes appearing slightly rough under oil immersion. This strain produces elliptical, usually large, white to pale buff sclerotia (125 - 300 × 750 - 2250 μ).

Conidiophores	653	- 1892 × 6.0 - 15.0 μ.
Conidial heads	51.5	- 137.5μ.
Vesicles	13.7	- 34.5 μ dia.
Metulae	.. 7.5	- 10.5 × 2.0 - 2.8 μ.
Phialides	.. 6.5	- 12 × 2.5 - 3 μ.
Conidia	.. 2.0	- 3.0 μ dia.

A. Sulphureous: Strain No. 2

Fast growing strain (3.5 cm. in diameter at a temperature of 75°F.±2°F. by single spore inoculation), with light yellow conidial heads which gradually change to yellow and after three to six

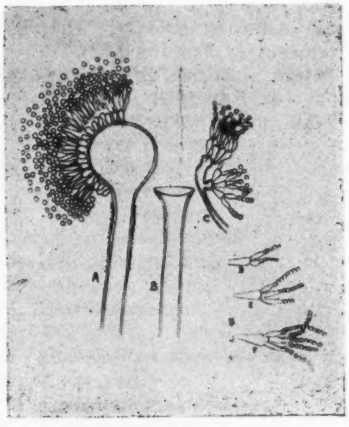

Fig. 1.—(*a*) Conidial head; (b) funnel-shaped conidiophore ends; (c) small conidial heads; (d-f) metulae and phialides.

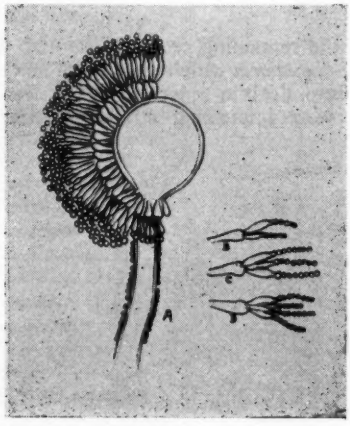

Fig. 2.—(*a*) Conidial head; (*b*) metulae and phialides.

days to ochraceous shade. There is regular zonation in the growth and some cottony aerial mycelium is found at places. No colouration on the reverse of the tube when young but brownish colouration seen in old cultures. Conidial heads are quite large and easily visible to the naked eye. Large, globose vesicles present. Conidiophores appreciably long (1479 - 2408 × 12.9 17.0 μ) with pitted and granulated walls (Fig. 2a). Metulae bearing two to four phialides (Fig. 2 b, c, d,); conidia globose to sub-globose, small and smooth. This strain does not produce sclerotia.

Conidiophore		1479 - 2408 × 12.9 - 17.0 μ.
Conidial heads		47.5 - 70.0 μ dia.
Vesicles	..	24.0 - 35.5 μ dia.
Metulae	..	9.5 - 12.0 × 2.8 - 3.8 μ.
Phialides	..	5.7 - 11.5 × 2.0 - 2.8 μ.
Conidia	..	2.0 - 3.0 μ dia.

A. Sulphureous: Strain No. 3

Slow growing strain (1.5 2 cm. in diameter at a temperature of 75° F.±2°F. by single spore inoculation); colonies on Czapek's agar powder with ochraceous colour. Conidial heads easily seen with the naked eye. Conidiophores brownish, short and rough, conidiophores occasionally curved below the conidial head. Vesicles globose to flask shaped (Fig. 3 a). The number of phialides

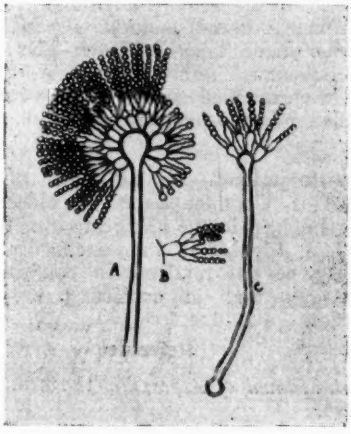

Fig. 3.—(*a*) Conidial head; (*b*) metulae and phialides; (*c*) small conidial heads.

attached to the metulae is usually four (Fig. 3b); very small conidial heads occasionally found (Fig. 3c). Conidia are usually smooth but fine roughening is occasionally found. White to pale buff sclerotia present (500 - 875 × 375 - 625 μ).

Conidiophores		498 - 722.5 × 6.8 - 10.0 μ
Conidial heads		28.5 - 60.0 μ dia.
Vesicles	..	20.0 - 30.5 μ dia.
Metulae		5.7 - 9.5 × 2.0 - 3.0 μ
Phialides	..	5.7 - 10.0 × 2.0 - 2.8 μ
Conidia	..	2.5 - 3.0 μ dia.

A. Sulphureous: Strain No. 4

Slow growing strain (3 cm. in diameter at a temperature of 75°F.±2°F. by single spore inoculation), colonies on Czapek's agar with very compact visible conidial heads of ochraceous shade. The reverse of the tube is brownish at places in old cultures. Conidiophores are short with fine roughening. Vesicles are large and globose (Fig. 4 a). Metulae bearing 2-8 phialides (Fig. 4 b-e). Conidia very small, globose, smooth but occasionally fine roughening is seen. This strain does not produce sclerotia.

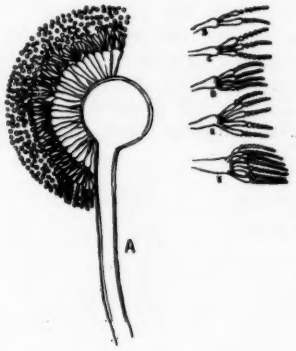

Fig. 4.—(*a*) Conidial head; (*b-e*) metulae and phialides.

Conidiophores		275 - 1376 × 24.0 - 58.5 μ.
Conidial heads		57.0 - 85.5 μ dia.
Vesicles	..	25.9 - 43.0 μ dia.
Metulae	..	7.6 - 15.0 × 3.8 - 5.7 μ
Phialides	..	6.0 - 11.5 × 1.9 - 2.4 μ
Conidia		1.9 - 3.0 μ dia.

A. Sulphureous: Strain No. 5

Slow growing strain (2 cm. diameter at a temperature of 75°F.±2°F. by single spore inoculation); colonies on Czapek's agar powdery, ochraceous in colour; the reverse of the tube is usually brown and the surface is very much wrinkled in old cultures. Conidial heads compact so that details of vesicles and phialides are not clearly visible. The conidiophores are wavy and sometimes bent below the conidial heads. Conidiophores are short,

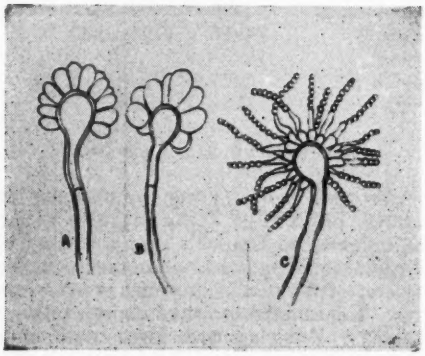

Fig. 5.—(*a*) Conidial head ; (*b-c*) baloon-like metulae.

finely roughened and slightly yellowish in the outer layer. (Fig. 5c). Vesicles small, globose or elliptical in shape. Metulae usually bear one phialide each. In the old cultures the phialides shed away leaving the metulae which bulge and form baloon-like structures (Fig. 5a and b). The old conidial heads with metulae are dark brown in colour. Conidia are globose, small and smooth walled. No sclerotia have been noted in this strain.

Conidiophores	..	172.0 - 1720 × 3.5 - 6.0 μ.
Conidial heads		34.5 - 68.5 μ. dia.
Vesicles	..	17.0 - 27.5 μ. dia.
Metulae	..	6.5 - 9.5 × 3.0 - 5.5 μ.
Phialides	..	7.5 - 13.5 × 2.0 - 3.5 μ.
Conidia	..	2.0 - 3.0 μ. dia.

Discussion

Measurements of the spores show that they are constantly small not exceeding more than 3μ in diameter. They are usually smooth, occasionally with fine roughening. According to Thom and Raper[1] these strains are to be placed unde· subseries Glaber. The sclerotia formation is found only in the strain No.1 and No. 3. In strain No. 1 the number of sclerotia is very poor and they are usually large (1250-3000×750-2250μ.) with white to pale buff colour. In strain No. 3, the sclerotia are not abundant and are of white to pale buff colour.

All the above strains are members of the *Aspergillus ochraceous* group. Dr. J. J. Elphick of the Commonwealth Mycological Institute, who very kindly confirmed the identification, is of the opinion that too much emphasis should not be placed on the colouration of the conidial heads. Dr. Elphick further noted that in all these strains and particularly No. 1 and No. 2 the heads are distinctly yellow when young (3-6 days old) but darken more or less rapidly on maturity to distinctly ochraceous shades.

This character would imply that these strains should belong to ocharaceus group proper, under *Aspergillus sulphureous* Fres.

The variation in colony appearance which exists between these strains is quite comparable with what is described for the species, and the same applies to the sclerotia, and also the microscopic details. Only their darker colour delimits them from the species as treated by Thom and Raper but this difference is not sufficient to justify either being squeezed into another species in the group or a new species being erected for them.

Conclusion

From the above description it is clear that the classification of the ochraceous group, primarily on the basis of the colour of conidial heads is not always found to be satisfactory. *Aspergilli* having ochraceous shades may be better placed in the sulphureous series rather than in the ochraceous series when examined minutely. The importance of detailed microscopic examination is thus obvious.

Acknowledgement.—We are much obliged to th› Director, Commonwealth Mycological Institute, for his help in the idetification of fungi and to Dr. J. J. Elphick of the same institute for the great help he has rendered in confirmation of identifications and in correcting the original manuscript.

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SHORT COMMUNICATIONS

STUDIES IN THE RELATIONSHIP BETWEEN VISCOSITY AND MOLECULAR STRUCTURE

Part VI.—Evidence for Fine Structure in the Steps Observed in the Energy of Activation of Viscous Flow in Ethylene Glycol

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(Received June 15, 1960)

In previous communications of this series, well-defined steps were observed in the curves for temperature variation of activation energy of viscous flow in ethylene glycol[1] and water.[2] For water, the steps were found to be sharp (Fig. 1, lowest curve), while for ethylene glycol the drop from one step to the next was gradual, extending over about 5°C. (Fig. 1, uppermost curve). In an effort to resolve this discrepancy, it was considered worthwhile to examine the effect of reducing the measuring interval of 2.5°C. previously used for the measurements on ethylene glycol. This interval has been reduced to 1°C., and some preliminary results obtained with a redistilled commercial grade glycol are presented in this communication.

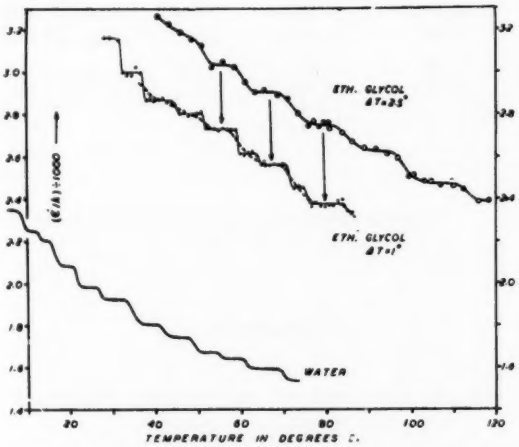

Fig. 1.—Showing the previously obtained data for ethylene glycol (large circles, uppermost curve) and for water (lowest curve), together with the now reported data obtained for ethylene glycol with a smaller measuring interval $\Delta T=1°C$. (small circles, middle curve ; the ordinates being displaced downward by 0.4 unit).

The experimental arrangement and other details are the same as in the previously reported investigation on ethylene glycol, except that a better thermostat was used and the ballast bottle device for prevention of evaporation used in the measurements on water[2] was also incorporated. The viscosity was obtained from time of flow measurements made every 1°C., five degrees being covered with one setting of the calibrated Beckmann thermometer.

The results obtained with rising and falling temperature sequences, together with the mean values of $(\varepsilon'/k) \div 1000$, obtained at one-degree intervals in the range of 28°C. to 86°C. are given in Table 1. The means are plotted as small solid circles in Fig. 1 (the ordinates having been shifted downward by 0.4 units); the thin continuous line shows the best graph that can be drawn through these experimental points, the standard deviation of which is seen to be about 0.004 from a comparisonof the readings taken with rising and falling temperature sequences. The graph shows a succession of sharp steps occurring at slightly irregular intervals of nearly 6°C., which is to be contrasted with the 12°C. interval found in the previous results obtained by Rauf and Qurashi,[1] and shown in Fig. 1 by large (solid and hollow) circles.

The striking differences between the two graphs can be resolved by smoothing the new curve (for the commercial grade) over a range of 2.5°C., this being the value of the measuring interval used in the earlier experiments. This smoothed graph is shown approximately by the thick broken line curve in Fig. 1. This curve runs closely parallel to the earlier results of Rauf and Qurashi plotted in the upper curve, thus showing agreement between the two sets of results as regards both the temperatures at the steps and energy values of the flat portions. This agreement for the experiments on two grades of glycol with two different thermostats can be taken as proof that (i) the phenomena being observed are essential properties of the

TABLE 1.—VALUES OF ACTIVATION ENERGY ε'/K OF ETHYLENE GLYCOL (COMMERCIAL GRADE) OBTAINED WITH MEASURING INTERVAL $\Delta T = 1$ °C.

Mean temperature (°C.)	$\frac{\varepsilon'}{k} \div 1000 = -\frac{T^2}{1000} \times (\Delta \ln \nu / \Delta T)$ Heating sequence	Cooling sequence	Mean	Mean Temperature (°C.)	$\frac{\varepsilon'}{k} \div 1000 = -\frac{T^2}{1000} \times (\Delta \ln \nu / \Delta T)$ Heating sequence	Cooling sequence	Mean
28.40	3.563	3.562	3.562±0.000	60.00	3.014	3.018	3.016±0.002
29.60	3.559	3.563	3.561±0.002	61.00	3.005	3.013	3.009±0.004
31.70	3.551	3.548	3.550±0.002	62.00	3.021	3.025	3.023±0.002
32.70	33.98	3.400	3.399±0.001	63.00	3.025	3.033	3.029±0.004
33.80	3.386	3.387	3.386±0.000	64.00	3.012	3.004	3.008±0.004
34.90	3.385	3.389	3.387±0.002	65.00	2.972	2.964	2.968±0.004
35.90	3.425	3.428	3.426±0.002	66.00	2.963	2.962	2.962±0.001
36.90	3.400	3.396	3.398±0.002	67.00	2.960	2.966	2.963±0.003
38.10	3.265	3.272	3.268±0.004	68.00	2.965	2.975	2.970±0.005
39.00	3.270	3.274	3.272±0.002	69.00	2.960	2.974	2.967±0.007
40.00	3.256	3.268	3.262±0.006	70.00	2.973	2.967	2.970±0.003
41.00	3.268	3.276	3.272±0.004	70.50	2.968	2.965	2.967±0.001
42.00	3.280	3.276	3.278±0.002	71.00	2.962	2.956	2.959±0.003
43.00	3.255	3.261	3.258±0.003	72.60	2.845	2.858	2.852±0.006
44.00	3.260	3.251	3.256±0.005	73.70	2.875	2.857	2.866±0.009
45.00	3.245	3.250	3.248±0.002	74.70	2.856	2.857	2.856±0.000
45.00	3.202	3.208	3.205±0.003	75.70	2.810	2.807	2.808±0.001
47.00	3.208	3.220	3.214±0.006	76.60	2.768	2.761	2.765±0.003
48.00	3.191	3.200	3.196±0.004	77.70	2.777	2.790	2.784±0.006
49.00	3.196	3.204	3.200±0.004	78.90	2.769	2.770	2.770±0.000
50.00	3.203	3.213	3.208±0.005	79.90	2.767	2.763	2.765±0.002
51.00	3.216	3.212	3.214±0.002	80.90	2.771	2.775	2.773±0.002
52.00	3.134	3.142	3.138±0.004	81.90	2.782	2.763	2.773±0.010
53.00	3.140	3.136	3.138±0.002	82.90	2.780	2.804	2.792±0.008
54.00	3.128	3.138	3.133±0.005	83.90	2.804	2.802	2.803±0.001
55.00	3.131	3.119	3.125±0.006	85.30	2.742	2.749	2.746±0.003
56.00	3.128	3.140	3.134±0.006	86.30	2.741	2.738	2.740±0.001
57.00	3.133	3.127	3.130±0.003				
58.00	3.125	3.130	3.128±0.002				
59.00	3.115	3.123	3.129±0.004				

Note: Standard deviation for the range 28° to 59°C. is ±0.004

Note: Standard deviation for the range 60° to 86° C. is ±0.004

ehtylene glycol and (ii) the steps are influenced only in small measure by the impurities (in the two grades of glycol) or by the specific vibrations, etc., of the two thermostats. We may thus expect that the refined experiments on purified ethylene glycol using the measuring interval of 1°C. will yield results essentially similar to those of the middle curve of Fig. 1, i.e. a series of sharp well-defined steps like those observed in water.

These experiments are in hand and their results and interpretations will be communicated separately in a fuller paper.

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TWO NEW SPECIES OF GENUS CURVULARIA

Curvularia Siddiqui Sp. novo and Curvularia Ellisi Sp. novo

S. IFTIKHAR AHMAD AND M. SAYEED QURAISHI

Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi

(Received May 20, 1960)

During the work on atmospheric fungi in Karachi, two species of the genus *curvularia* were collected and were confirmed as new species by Dr. Ellis of the Commonwealth Mycological Institute.

Few species of the genus *curvularia* have been described from the sub-continent of Pakistan and India, and those described have been isolated either from the soil or seeds. The present species have been isolated from air and seem to differ from the known species of the genus described from this country and elsewhere, [1-4] and hence are described as new species.

1. Curvularia Siddiqui sp. novo

Colony when grown on Czapek's agar pH 6 grows fast and is buff-coloured in the beginning, changing to olive in the absence of conidial formation and finally dark grey or black when conidia are formed. Older colonies are covered with greyish mycelium which spreads on the surface.

Mycelium.—With profuse branching, the branches closely interwoven and twisted, forming a tough leathery layer on the surface of the medium. Hyphal diameter 3.44-7 μ, chlamydospores frequently seen in cultures, stromata not seen.

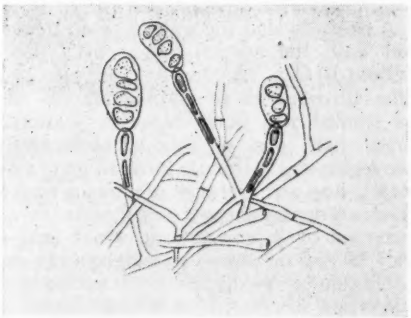

Fig. 1.—Camera lucida drawing of short, unbranched conidiophores of *Curvularia Siddiqui* bearing solitary conidium.

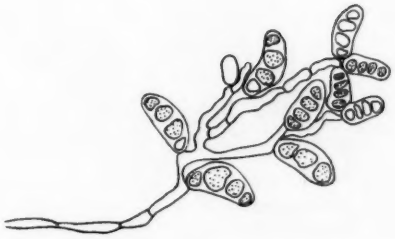

Fig. 2.—Camera lucida drawing of ong, dichotomously branched conidiolphore of *Curvularia Siddiqui* with many conidia.

Conidiophores.—Multiseptate and of two types; one, short and unbranched 34-172μ, the cell at the tip wider and club-shaped, 5.6-6.88μ in diameter, bearing a solitary conidium (Fig. 1), the other type of conidiophore is long and dichotomously branched with short alternate bends bearing a conidium at each bend; the tip of the cell bearing conidium is club-shaped, the length of the conidiophore is very variable, 206-413μ or more, the width is 5.160-7.74μ (Fig. 2).

Conidia.—Four-celled, slightly curved, second and third cell larger and darker than others. The size of the conidium is 20-38×10.5-17.2μ.

The shape and structure of the conidiophores differentiate this species from other related species.

We have great pleasure in dedicating this species to Dr. Salimuzzaman Siddiqui to whom we are greatly indebted for guiding us throughout the progress of this work.

2. Curvularia Ellisi sp. novo

Colony, when grown on Czapek's agar pH 6, velvety, fast growing, light green in the beginning, darkening with age and finally turning black. Older colonies covered with a whitish fluffy mycelium restricted to patches on the surface of the colony.

Mycelium septate, irregularly branched, terminal cells of the branches elongated; hyphal diameter 3.44-6.88μ. Chlamydospores frequently seen in cultures; stromata not seen.

Conidiophores multiseptate, usually short and erect or reclining, geniculate at the tip, usually 35-258 μ in length, some times much longer, 5.1-8.6μ in diameter at the tip (Fig. 3); spores forming clusters at the tip and also borne along the length of the condiophore. Diameter of the basal cell of the conidiophore 2.5-6 μ.

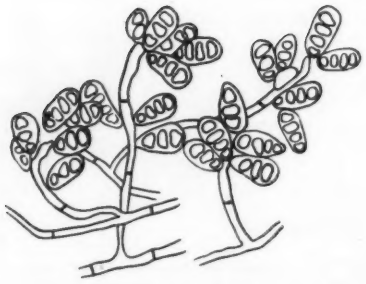

Fig. 3.—Camera lucida drawing of conidiophores of *Curvularia Ellisi*.

Conidia curved, four celled, second and third cells from the base darker and wider than others, conidia measuring 18.92-26.3×9.6-13.43 μ.

The species differs from the related species of *Curvularia lunatum* in having branched conidiophores, and in the size of the conidia and conidiophores.

We have great pleasure in dedicating this species to Dr. Ellis of Commonwealth Mycological Institute to whom we are greatly indebted for confirming it as a new species and for going through the manuscript of this paper.

Locality.—Karachi, Pakistan, from air at a height of 10 feet on 10th December, 1956.

Collected by :— S. Iftikhar Ahmad.

Type specimen at these Laboratories and Commonwealth Mycological Institute, Surrey.

Acknowledgement.—We are much obliged to Dr. J. C. F. Hopkins, Director, Commonwealth Mycological Institute, for his help in indentification of fungi. Special thanks are due to Dr. Ellis of the same institute for his confirmation of the above species as new and for valuable suggestions, The authors also thank Dr. Siddiqui for his encouragement.

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RESTING HABITS OF ANOPHELES SPECIES IN MYMENSINGH DISTRICT, EAST PAKISTAN

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(Received February 2, 1960)

Study of the resting habits of vector species is essential before large scale indoor spraying operations with residual insecticides are carried out for the control of Malaria in a given area. The WHO Malaria Control Demonstration team[1] to Pakistan, therefore, studied the resting habits of local anopheles species in Ishwarganj Thana in Mymensingh District before starting spraying operations. The present paper is based on the work done during this period.

In papers previously published it was stated that the vector, *A. philippinensis* was found in somewhat unusual situations. "It does not rest in situations where other anophelines are normally to be found, such as the ceiling, thatch, cobwebs, etc. It generally rests on the wall very close to the floor of the room, within a foot or a foot and a half from floor level, being only very rarely found at higher levels"—Iyengar.[2]

The author did not find this peculiar behaviour on the part of *A. philippinensis* in the area but on the other hand noted that all of the commoner species of anopheles show a preference to rest on the lower half of walls in bedrooms and that, with the exception of *A. annularis*, they exhibit the same habit in cowsheds.

Regular collections were made in bed rooms and cattle sheds in the area: an arbitrary limit of five feet was chosen because this height in a majority of cases divided the walls into an upper and a lower half, the normal height of a bedroom being about 10 feet. A careful study also showed that the distribution of individuals of all the species studied was fairly regularly scattered on the lower half, and no special preference was thus shown for any particular location up to a height of five feet, nor was any such preference noticeable in individuals resting above a height of five feet, with the exception of *A. annularis*, in which case some tendency to rest on straws projecting from ceiling was noticeable in cowsheds.

The present paper is a statistical analysis of the data obtained. The results from bedrooms and cowsheds have been summarized in Tables 1 and 2,

respectively. Other entomological observations have been reported in the Final Comprehensive Report Pakistan, East Bengal Malaria Control Demonstration Team Mymensingh District.

Mosquitoes in Bedrooms

The values of P in the table range from 0.18 to 0.40 with $\bar{P}=0.3006$, thus indicating a definite preference of the mosquitos to rest below 5 feet. Assuming that all the species rest independently of height, i.e. the proportion resting above 5 feet and below 5 feet is the same, we may apply the x^2 test, viz.

$$x^2=\frac{1}{\bar{p}\,\bar{q}}[\Sigma(ap)-n\bar{p}]$$

with (6−1) (2−1) degrees of freedom where $n=1568$, $\bar{p}=1568/3649=.3006$, $q=1-\bar{p}=0.6994$, we get with five degrees of freedom,

$$x^2=\frac{1}{.3006\times .6994}(492.806-471.2715)=102.441.$$

This is almost ten times the value of x^2 with 5 d. f. 11.070 (Fisher's table), thus showing that in the above data variations from species to species are statistically significant.

The habits of individual species can now be analysed in the light of the data obtained. The normal test is applied, since the sample is considered to be large enough to conform to the normal distributions, and we calculate

$$\sigma=\sqrt{npq}$$

where n= total number, $p=q=\frac{1}{2}$.

For *A. philippinensis* we have n=994, number captured above 5 ft.=269, number captured below 5 ft.=725, and deviation=456, yielding

$$\sigma=\sqrt{994\times\tfrac{1}{2}\times\tfrac{1}{2}}=\quad 15.8.$$

The observed deviation being more than 14 times the calculated value of σ, the result is significant, establishing that *A. philippinensis* has a tendency to rest below 5 ft. in bedrooms.

Similarly for *A. aconitus* we have

$$\sigma=\sqrt{1138\times\tfrac{1}{2}\times\tfrac{1}{2}}=16.867$$

while the value of observed deviation is 366 i.e. about 22 times the calculated value of σ this establishes that *A. aconitus* has a tendency to rest below 5 ft. in bedrooms.

Applying similar test to *A. annularis* we find the observed deviation of 124 is more than 7 times the calculated value of σ which is 16.031, and in the case of *A. vagus* we find the observed deviation of 301.5 to be more than 13 times the calculated value of σ, i.e. 22.071. In these two species also, the marked tendency for *A. vagus* to rest below a height of 5 feet in bedrooms is therefore established.

Mosquitoes in Cowsheds

The number of anopheline mosquitoes caught in cow sheds according to height have been shown in Table 2.

Here again on the assumption that all the species rest independently of height, i.e. the proportion resting above 5 feet and below 5 feet is the same, the x^2 test gives

TABLE 1.—THE NUMBER OF ANOPHELES SPP. CAUGHT ABOVE AND BELOW A HEIGHT OF FIVE FEET IN BEEDROOMS.

Anopheles species	Caught above 5 feet =a	Caught below 5 feet =a′	Total =a+a′	Proportion caught above 5 ft. $p=\frac{a}{a+a'}$	a.p.
Philippinensis:	269	725	4994	.2706	72.8
Aconitus	203	935	1138	.1784	36.2
Annularis	390	638	1028	.3794	148.0
Hyrcanus	20	50	70	.4000	8.0
Vagus	673	1276	1949	.3453	223.4
Rest(pooled)	13	25	38	.3421	4.4
	1,568	3,649	5,217	$\bar{p}=.3006$	492.8

TABLE 2.—THE NUMBER OF ANOPHELES SPECIES CAUGHT ABOVE AND BELOW A HEIGHT OF 5 FT. IN COWSHEDS.

Anopheles species	Caught above 5 feet a	Caught below 5 feet a′	Total	Proportion caught above 5 ft. $p=\frac{a}{a+a'}$	a.p.
Philippinensis	201	298	499	.4028	80.9
Aconitus	1179	2297	3476	.3392	399.8
Annularis	20296	14239	34535	.5877	11927.8
Vagus	2426	2844	5270	.4603	1116.7
Hyrcanus	812	1425	2237	.3630	294.7
Barbirostris	315	273	588	.5357	168.7
Rest	244	197	441	.5533	135.0
Total	25473	21573	47046		14123.9

$$x^2 = \frac{1}{.54145 \times .45855} \cdot (14123.9752 - 13792.222)$$
$$= 610{\cdot}7632 \text{ with 6 d.f.}$$

The value of x^2 with 6 d.f. from Fisher's table is 12.592. The assumption is, therefore, discredited and we find that species and the heights on which they rest are not independent, i.e. species have different proportions resting above and below 5 feet. As in the case of distribution in bedrooms, here also the samples are considered to be large enough to conform to the normal distribution and normal test is applied to individual species.

Applying the normal test to *A. aconitus* we have $\sigma = 29.5$, the observed deviation being 19 times the value of σ the result is highly significant showing a tendency for *A. aconitus* to rest *below* a height of 5 feet in cowsheds.

In the case of *A. annularis*, P is 0.5877, i.e. greater than $\frac{1}{2}$, and we get $\sigma = 92.9$, the observed deviation, 3028.5 being thus about 33 times the calculated deviation. This shows a marked tendency for *A. annularis* to rest *above* 5 feet in cowsheds. From similar calculations for *A. hyrcanus* and *A. vagus* the value of σ is calculated to be 23.6 and 36.3, respectively. The observed deviations in these cases are 306.5 and 209, respectively, or 13 and 6 times the calculated values of σ. Hence in both these cases there is a tendency to rest below 5 feet. This tendency, however, is more marked in the case of *A. hyrcanus* as compared with that of *A. vagus*.

A. philippinensis and *A. barbirostris* were not caught in sufficient numbers to justify the application of normal test, and numbers captured are, therefore, given without analysis. The data do not show any special tendency in *A. philippinensis* to rest near the floor, nor did *A. philippinensis* show any significant difference in its resting habit in bedrooms as compared with that of other anophelines.

The tendency to rest on lower half of the walls in bedrooms is found among all the anopheline species commonly found in the area. The proportion resting above a height of five feet is however not negligible but on the contrary is considerable. In cowsheds also all the common species, except *A. annularis*, show a tendency to rest below a height of five feet. However, the commonest species in cowsheds, *A. annularis*, rests predominantly on the upper half of the walls. *A. annularis* is thus the only species which shows different resting habits in bedrooms and cowsheds.

In the light of the data provided in this paper, it would appear necessary that in any spraying operations the entire inner structures including the ceiling have got to be sprayed to achieve satisfactory control.

Acknowledgements.—The author wishes to express his thanks to Dr. G. Gramiccia, leader of the WHO team, and other senior members and members of the entomology section of the WHO team, also to the villagers through whose cooperation this study was made possible. Thanks are also due to Mr. A.B.M. Lutful Kabir of the Pakistan Council of Scientific and Industrial Research for his help in the statistical analysis of the data. The author further wishes to express his deep sense of gratitude to Col. M. K. Afridi for going through the manuscript and making valuable suggestions.

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BOOK NOTICES

An Introduction to Co-ordination Chemistry. D.P. Graddon. 120 pp. Pergamon Press, London, 1960. Price 25s. ($4.00).

In this important volume Dr. Graddon, Senior Lecturer in Inorganic Chemistry at the University of New South Wales, gives an authoritative introduction to the subject of co-ordination chemistry. After an interesting historical introduction to the subject, dating from the earliest recorded co-ordination compound, (prussian blue, obtained by the artists' colour maker Diesbach, in Berlin, during the first decade of the eighteenth century) to present day knowledge, Dr. Graddon discusses the latest theory and practice. A bibliography as a guide to further study is included.

This volume will be welcomed by all requiring a thorough and scholarly introduction to the subject.

A Laboratory Manual of Analytical Methods of Protein Chemistry (Including Polypeptides). P. Alexander and R.J. Block, Three volumes. Pergamon Press, London, 1960.

In the last decade great advances have been made in the experimental methods available for studying the chemical constitution, structure and physical properties of proteins. This laboratory manual provides instructions on how to carry out these different analyses in detail so that reference to other publications is unnecessary.

The researcher faced with a problem in protein chemistry can find in this book the best method for the job and instructions on how to do it. All the contributors have used the methods they describe in their own laboratories and are fully conversant with all aspects of the technique.

Theoretical treatments are given briefly and only to the extent necessary for working out the results and to appreciate the uses and limitations of the method.

In the first volume, separation and isolation procedures are discussed; the second volume concerns its analysis and reactivity, and the third volume with the measurement of the macromolecular properties of proteins.

Advances in Polarography. I.S. Longmuir. Approx. 1200 pp. in 3 volumes. Pergamon Press, London, 1960. Price £ 15/- ($45.00) per set of 3 vols.

These important volumes contain the authoritative proceedings of the Second International Congress held at Cambridge to honour the seventieth birthday of Nobel Laureate Jaroslav Heyrovsky, whose genius created Polarography and fostered its development in many fields.

The multiplicity of outstanding contributions to this conference by leading experts throughout the world ensures that these volumes represent a more up to date account of the state of polarography than could be presented in a work by a single author.

The programme was organized in six sections: instrumentation, theory and kinetics, analytical and industrial applications, fundamental studies, biological and medical applications, and a final section containing those papers which do not fit itno any of the other categories.

All interested in this increasingly important field will welcome these valuable volumes.

Analytical Chemistry of the Rare Earths. R.C. Vickery, 143 pp. Pergamon Press, London, 1960. Price 40s. ($6.50).

Analytical Chemistry of the Rare Earths is the first volume to appear devoted specifically to the analytical problems encountered in dealing with the rare earths. It covers all phases of the problems from decomposition of the analytical sample to ultimate element determination. Gravimetric, volumetric, spectrophotometric, spectrographic x-Ray and polarographic techniques are all considered with the special requirements of each technique to analysis of these elements. This authoritative book is written not only for the specialist analyst but, by using the imperative mood for procedures, is particularly adaptable for graduate and undergraduate students of analytical chemistry. Hitherto the literature on analysis for the rare earths has been widespread in journals and little attempt has been made to correlate procedures and define the best method of operation. These deficiencies have been corrected with this book which has critically surveyed the literature and, with the author's long experience in this field presents what might be

considered the text book or source book of its subject. All applicable methods of analysis have been considered and the literature has been covered up to date.

Dr. R.C. Vickery, who has been working in the rare earth field for nearly 20 years, is author of the "Chemistry of the Lanthanons" and "Chemistry of Yttrium & Scandium," and some 75 professional papers on the rare earth elements.

Human Growth. J. M. Tanner and J.R. Napier. 128 pp. Pergamon Press, London, 1960. Price 30s. ($ 5.00).

This fascinating and important book contains the authoritative proceedings of a Symposium organized by the Society for the Study of Human Biology, held at the University College, London. The aims of the Society are to advance the study of the biology of human populations and of man as a species, in all its branches, particularly human variability, human genetics and evolution, human adaptability and ecology.

The success of the meeting can be judged by the high standard of the papers contained in this volume, and testifies to the tremendous increase in interest on the part of anatomists, zoologists, anthropologists and paediatricians in this challenging field.

Organic Sulfur Compounds. N. Kharasch. 400 pp. Pergamon Press, London, 1960. Price £7/- ($20.00).

Organic Sulfur Compounds contains brief critical essays, each with a bibliography, on technical and theoretical aspects of organic sulfur chemistry. There is a special appendix giving 'capsule extracts' of other studies in organic sulfur chemistry of major interest.

This is the first of a series of volumes whose purpose is to present brief, authoritative, well-documented articles on current aspects of organic sulfur compounds. In this volume are articles on physical aspects of organic sulfur compounds, studies of reaction mechanisms of organic sulfur compounds, progress in synthesis and reactions of selected groups of organic sulfur compounds and biochemical aspects of selected organic sulfur compounds. Industrial as well as theoretical interests are included.

Organic Sulfur Compounds will be of considerable value as a reference book for university courses, and as a collateral text for graduate courses, whilst to a large group of readers whose work involves the chemistry of petroleum, rubber, and pharmaceuticals, and theoretical and synthetic organic chemistry, the volume will be extremely useful.

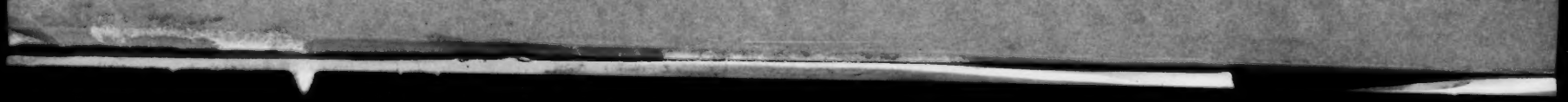
The Use of Organic Reagents in Inorganic Analysis. A.I. Busev and A.G. Polianskii. 76 pp. Pergamon Press, London. Price 21s. ($3.50).

The Use of Organic Reagents in Inorganic Analysis surveys and summarises the present state of knowledge in this currently active field.

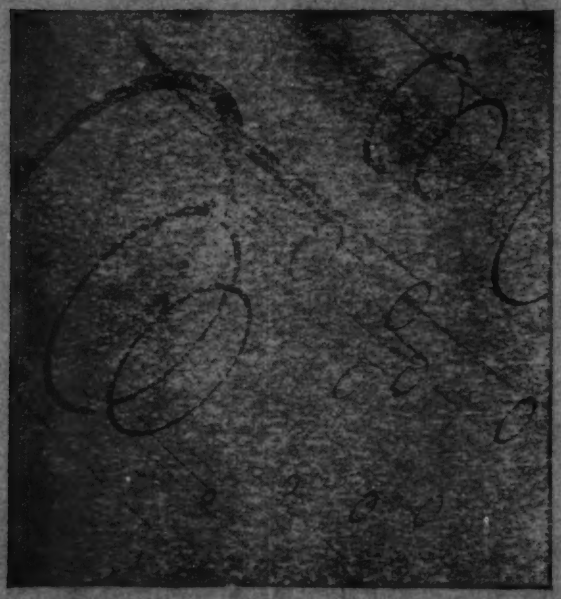
During the 70 years which have elapsed since the discovery of a method of determining cobalt by means of α-nitroso-β-naphthol, several thousand papers have been published on the application of organic reagents in inorganic analysis. The considerable interest taken in these organic reagents is due to the high analytical value of their properties. The small solubility and large molecular weight of the metallic compounds which they form favour their use in a gravimetric analysis. In addition, the high intensity of colouration of numerous reaction products of different ions with organic reagents makes the latter irreplaceable for the determination of trace quantities of substances by the photometric method.

No review classifying the numerous uses of organic reagents in inorganic analysis in recent years has been published, although the need for such a review has been developing for a long time. In drawing up the present review, papers which in the authors' opinion were of principal interest have been used. Many papers on complexones, and also papers on the use of organic reagents in chromatography, are not included (as suitable reviews have recently been published).

This most informative and useful volume will be invaluable to all workers in this sphere.



Through thick and thin Pyrex



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